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THE VALIDITY AND MORPHOLOGY OF TWO TRYBLIDIELLA SPECIES

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(WITH 4 FIGURES)

The lack of agreement in the literature on the taxonomic treatment of the genus *Tryblidiella* is perhaps due largely to our still meager knowledge of the natural relationships in this group of fungi. In his description of the genus *Tryblidiella,* Saccardo (**7**), included a group of Discomycetes having brown ascospores with two or more septa, and designated *T. rufula* (Spreng.) Sacc. as the type species. In 1892, Ellis & Everhart (**4**), reduced the genus *Tryblidiella* to a sub-genus of *Tryblidium.* Later, 1904, Rehm (**6**), included the one-septate brown spored species under *Tryblidiella,* sub-genus *Eutryblidiella,* and the three- to five-septate brown spored species under *Tryblidiella,* sub-genus *Rhydithysterium.* After reviewing the literature on the genus *Tryblidiella,* it is the opinion of the writer that those forms having brown three-septate ascospores belong in this genus as originally described by Saccardo.

Of this group, *Tryblidiella rufula* (Spreng.) Sacc., and *Tryblidiella fusca* (Ellis & Ev.) Rehm were the forms studied. *T. rufula,* a somewhat variable species, was described by Saccardo (**7**), in 1883. Later, 1889, material collected by Calkins near Jacksonville, Florida, was described by Ellis & Everhart (**3**), as *Tryblidium rufulum* Spreng. var. *fuscum* Ellis & Ev., because it

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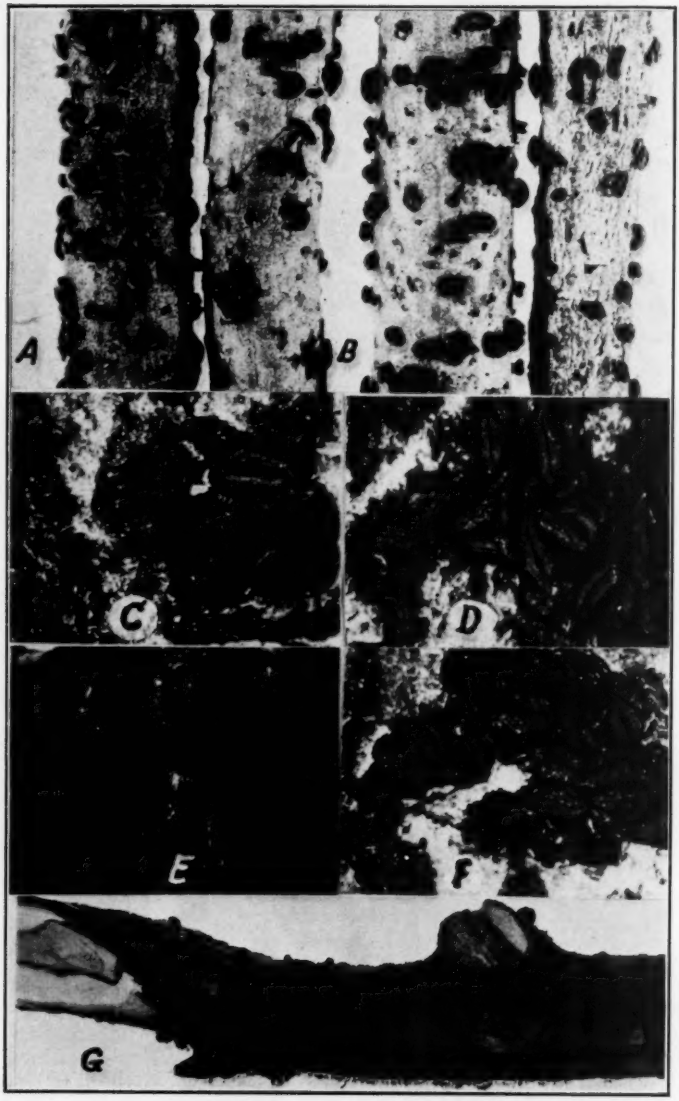

FIG. 1. (*A*), open and closed apothecia of *Tryblidiella rufula* on *Pistacia chinensis*, No. *12166;* (*B*), open and closed apothecia of *T. fusca* on *Pistacia chinensis*, No. *12167*. All × 2; (*C* and *D*), open and closed apothecia of *T. rufula* on *Pistacia chinensis*, No. *12166;* (*E* and *F*), open and closed apothecia of *T. fusca* on *Pistacia chinensis*, No. *12167*. All × 7. (*G*), apothecia of *T. fusca* on dying limb of *Pyrus chinensis*, natural size.

had a slate-colored hymenium and the lips of the apothecia very distinctly striate. In 1892, they (**4**) gave this fungus the specific rank of *T. fuscum* Ellis & Ev., on account of the clustered apothecia, slate-colored hymenium and the clavate-tipped paraphyses. In 1900, Rhem (**5**) changed the binomial to *Tryblidiella fusca* (Ellis & Ev.) Rehm. Later, 1904, he (**6**) reduced it to a variety of *T. rufula* (Spreng.) Sacc. As a result of the present studies, both *T. rufula* and *T. fusca* are considered entitled to specific rank. This conclusion is based on differences in some of their outstanding morphological characters in nature and in culture, as described below.

Although the apothecia of both species are commonly found together on the same specimen, they may be easily separated on the basis of certain macroscopic characters. The lips of the apothecia may or may not be transversely striate in *T. rufula,* but when present these striae are rather indistinct as compared with the very distinct striae of *T. fusca* (FIG. 1, *C, D, E, F*). Also, the brick-red hymenium of the brownish-black apothecia of the former is quite distinct from the slate-colored hymenium of the black apothecia of the latter. The brick-red hymenium of *T. rufula* is usually indistinct until the apothecia have been opened by moisture. When dry the sides of the apothecia of both species roll in so as to partly or almost completely cover the hymenium, and the measurements of both forms fall within the limits of 1–3.5 × .5–1 mm., but when moist the width of the apothecia of *T. fusca* may exceed that of *T. rufula* by .5 mm. The apothecia of *T. fusca* may be slightly more stipitate than those of *T. rufula,* and the lips of the former are slightly thicker than those of the latter. In other respects the apothecia of these two species are quite similar in that they may be scattered or cespitose, erumpent-superficial, suborbicular, elliptical, triangular or otherwise irregular from crowding (FIG. 1).

The two species are inseparable on the basis of known microscopic characters. Their asci are cylindrical, with abundant clavate-tipped paraphyses. The ascospores are uniseriate, oblong, three-septate, constricted at the septa, and reddish-brown in color (FIG. 4). In the present study 100 ascospores of *T. rufula,* from

Pistacia chinensis, No. *12166,*[1] and *Pyrus chinensis,* No. *12168,* measured *27–36* × 10–14 μ, averaging 31 × 11 μ, as compared with 30–35 × 10 μ, as given by Saccardo (**7**), and 24–30 × 10–12 μ as given by Ellis and Everhart (**4**). Also, 100 ascospores of *T. fusca,* from *Pistacia chinensis,* No. *12167,* and *Pyrus chinensis,* No. *12169* measured *27–37* × 10–14 μ, averaging 31 × 11 μ, as compared with 25–30 × 10–14 μ, as given by Ellis and Everhart (**4**).

Several single ascospore cultures of both species from various hosts were grown on cornmeal agar and potato-dextrose agar in test tubes and petri dishes. At first the mycelial growth from ascospores of both species was dirty-white in appearance, the mycelium of the *T. rufula* becoming light reddish-brown to mouse-gray, while that of *T. fusca* became deep reddish-brown to blackish-brown. In all cases the mycelium of *T. fusca* was darker than that of *T. rufula.*

Later several single ascospore cultures of both species were planted singly and in various combinations on cornmeal in flasks. After a period of approximately thirty days a few small dull white gelatinous masses were observed scattered over the surface of some of these cultures (FIG. *2 B*). Microscopic examination showed these masses to consist of numerous hyaline subglobose microspores 2–3 μ in diameter (FIG. 3 *E*), similar to those described by Shear (**10**). Further examination showed these small spores to be exuded from numerous locules, irregular in size and shape, resembling micropycnidia and embedded in a somewhat discontinuous stroma (FIG. 2, *C, D*). The microspores are borne on short conidiophores lining the inner surface of these locules. Microspores from ascospore cultures of both *T. rufula* and *T. fusca* were transferred to potato-dextrose agar in petri dishes. After a reasonable period of time they showed no signs of germination, thus they were considered to be made cells of the fungus, perhaps similar to the spermatia of certain other discomycetes, Laboulbeniales, and the rusts. The manner in which the microspores of these two *Tryblidiella* species are borne would compare more closely to the spermagonia of certain forms in the genus *Pycnopeziza*

[1] Numbers refer to specimens in the Florida Agricultural Experiment Station Herbarium.

FIG. 2. (*A*), mature apothecia of *Tryblidiella fusca* from single ascospore culture on cornmeal, × 7; (*B*), microspores exuding from micropycnidia of *T. fusca* from single ascospore culture on cornmeal, × 7. Freehand sections of pycnidia from single ascospore cultures on cornmeal; (*C*), micropycnidia of *T. rufula;* (*D*), micropycnidium of *T. fusca;* (*E*), macropycnidia of *T. fusca.* All × 200.

(**11**), than to similar structures of other discomycetes and ascomycetes where they are borne on conidiophores arising directly from the mycelium. Due to the lack of cytological evidence in these studies, nothing definite can be concluded concerning the function of these microspores at present. However, since mature apothecia developed in some of the single ascospore cultures of both species (FIG. 2 *A*), as well as in some of the flasks containing two ascospore cultures, it seems reasonable to assume that if these miscrospores are male sex organs, spermatization between compatible isolates is not necessary for the production of mature apothecia. In other words, single ascospore cultures of these two *Tryblidiella* species seem to be structurally hermaphroditic and self-fertile, as compared to single uninucleate ascospore cultures of such forms as *Sclerotinia Gladioli* (Massey) Drayton (**2**), and *Pleurage anserina* (Ces.) Kuntze (**1**), which are also structurally hermaphroditic but self-sterile. According to Shear (**10**) mature apothecia were also produced in a single ascospore culture of *T. Leprieuri* (Mont.) Sacc.

In most of the cornmeal cultures of *T. rufula* and *T. fusca* *Diplodia*-like pycnidia were also found embedded and associated with the micropycnidia in the same stroma (FIG. 2 *E*). Both pycnidia types were also produced in some of the test tube cultures. Spores from these *Diplodia*-like pycnidia of both species were one-septate, yellowish-brown, falling within the limits of 15–28 × 7–12 μ, the pycnospores of *T. rufula* averaged 23.3 × 9.6 μ, while those of *T. fusca* averaged 22.2 × 8.4 μ (FIG. 3, *B, C*). According to Shear (**10**) two of his collections on different hosts which agreed with the description of *T. rufula,* and which could not be separated on the basis of the morphology of their apothecia and ascospores, produced distinctly different pycnospores in culture, one type being broadly striate and resembling *D. natalensis,* in everything but size, the other type resembling pycnospores of *D. Mori.* Thus, he is doubtful which, if either, is true *T. rufula.* In the present studies none of the *Diplodia*-like spores from ascospore cultures of either *T. rufula* or *T. fusca* showed any striations similar to those of *D. natalensis.* Therefore, the absence of these markings on the spore wall, as well as the color, size and shape of these spores would distinguish them from the

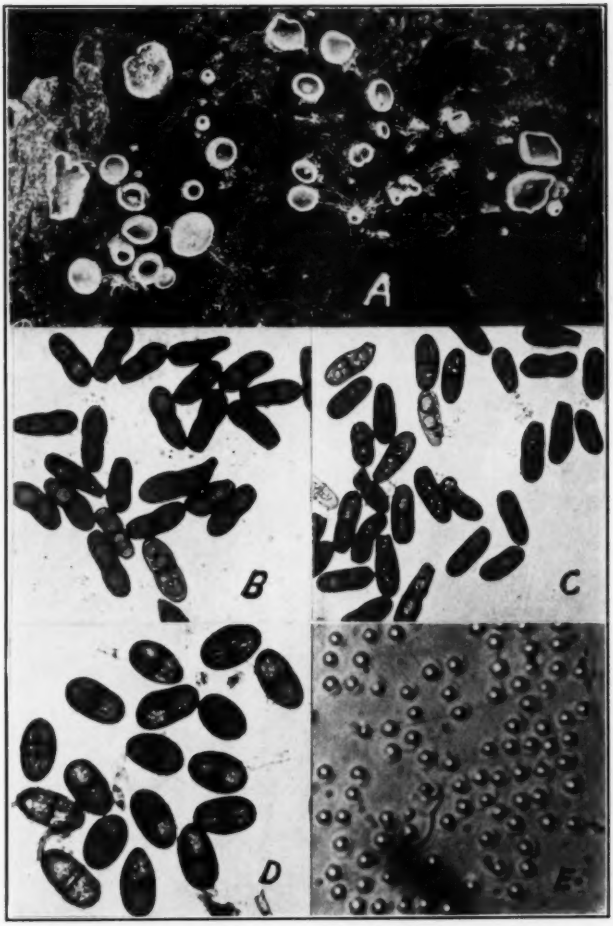

FIG. 3. (*A*), apothecia of *Cenangium Ravenelii* growing on apothecia of *Tryblidiella rufula* on *Pistacia chinensis*, No. *12170*, × 7; (*B*), pycnospores from single ascospore culture of *T. rufula* on cornmeal; (*C*), pycnospores from single ascospore culture of *T. fusca* on cornmeal; (*D*), pycnospores of *D. natalensis* from single pycnospore culture on cornmeal. All × 400; (*E*), microspores of *T. rufula* from single ascospore culture on cornmeal, × 1000.

typical spores of *D. natalensis*. The ratio of length to width of the pycnospores of *T. rufula* and *T. fusca* is greater than that in pycnospores of *D. natalensis*. The pycnospores of these two *Tryblidiella* species in culture are also more irregular in size and shape and more constricted at the septa than those of *D. natalensis* (FIG. 3 *B, C, D*). Also the few pycnidia found associated with, but probably not connected with, the *Tryblidiella* apothecia in nature do not produce spores comparing with those found in the present culture studies, but are of the *D. natalensis* type. The mycelial growth of several single pycnospore cultures of both *Tryblidiella* species could not be differentiated from that of the ascospore cultures. The mycelial growth of both *T. rufula* and *T. fusca* is very slow and the rate and color of the mycelial growth of both species in culture is quite distinct from that of *D. natalensis*.

Since these forms are, for the most part, considered to be saprophytic in nature, occurring on dead limbs of various trees, no attempt has been made in the present study to complete a list of the hosts on which they have been reported. In a few collection these two forms appeared to function as wound parasites, but there is no experimental evidence confirming their possible parasitism. As shown in figure 1 *G*, a pear limb is apparently being killed by *T. fusca*, which probably entered through a wound on a side branch. Collections of these two forms on various hosts have been deposited in the following herbaria: Florida Agricultural Experiment Station; New York Botanical Garden; Bureau of Plant Industry, Washington, D. C.

CENANGIUM RAVENELII ON TRYBLIDIELLA APOTHECIA

A Discomycete having small, cup-shaped, reddish-brown apothecia, and known as *Cenangium Ravenelii* (Berk. & Curt.) Sacc. (**8**), was found growing on apothecia of *T. rufula* and *T. fusca*. It was found on the apothecia of *T. rufula* in several collections (FIG. 3 *A*), but was found in only one collection of *T. fusca*. A few apothecia of this fungus appeared to be growing directly on the host bark. However, due to the lack of experimental evidence nothing definite can be concluded concerning its possible parasitism on *Tryblidiella* apothecia.

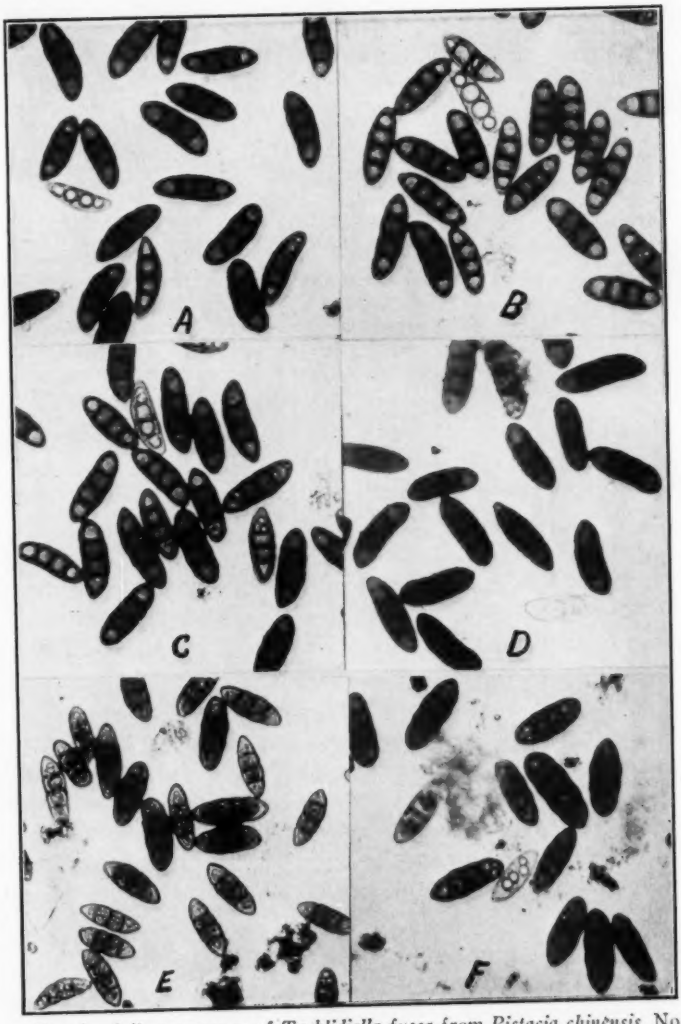

FIG. 4. (*A*), ascospores of *Tryblidiella fusca* from *Pistacia chinensis*, No. *12167*; (*B*), ascospores of *T. fusca* from *Pyrus chinensis*, No. *12166*; (*D*), ascospores of *T. rufula* from *Pyrus chinensis*, No. *12168*; (*E*), ascospores from single ascospore culture of *T. fusca* from *Pistacia chinensis*, No. *12167*; (*F*), ascospores from single ascospore culture of *T. rufula* from *Pistacia chinensis*, No. *12166*. All × 400.

This fungus has been reported from North America on *T. rufula,* and *T. nigrocinnabarina* (**9**). However, as far as known this is the first report of it on *T. fusca,* and also the first report of it on *T. rufula* and *T. fusca* from Florida.

DISCUSSION

The genetic connection of the different types of fruiting bodies here described as occurring in the life cycles of *T. rufula* and *T. fusca,* cannot be ascertained at present. Collections of these two forms made throughout the year have failed to reveal the presence of any pycnidial and spermogonial structures similar to those produced in pure culture. However, the presence of these structures may not be necessary for the reproduction of these forms in nature, since mature apothecia of both species were produced in single ascospore cultures.

According to our present ideas of classification, these two forms can be separated into distinct species by the morphological characters of the ascocarps. The conidial forms of *T. rufula* and *T. fusca* must be regarded as having little or no specific significance, at least for the present. The genus *Tryblidiella* has been insufficiently studied and, therefore, any scheme of natural classification applied to it must be regarded as tentative. Although the general character and appearance of the *Tryblidiella* pycnospores are similar to those in the genus *Diplodia,* yet, as Shear (**10**) has suggested, it seems rather remarkable that the pycnidial forms of such widely separated genera as *Tryblidiella* and *Physalospora* should be referred to the same form genus, *Diplodia.* A more thorough study of the characters of these pycnidial forms, however, may show sufficient differences to separate generically the species having such widely different perfect stages.

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HOMOTHALLISM IN PYTHIUM

T. C. VANTERPOOL

Investigations on the nature of the thallus in the Peronosporales have been confined to *Phytophthora* and *Peronospora,* in both of which complicated conditions exist within a single species. Studies on the sexual relations of species of *Pythium* with single zoöspore cultures have not been reported. Long cultural experience with the genus *Pythium,* however, has given no indication that heterothallism exists in the group. In spite, therefore, of investigating the obvious homothallism of *Pythium,* preliminary studies on single zoöspore cultures were undertaken. The majority of species of this genus are monoclinous, that is, the antheridia arise in more or less close proximity to the oögonium; in the diclinous species the antheridia arise from hyphae showing no traceable connection with the hypha bearing the oögonium or only extremely rarely. If heterothallic forms exist they would more likely be diclinous.

The work of Ashby (**1**, **2**, **3**), Narasimhan (**9**), Leonian (**7**) and others shows that in *Phytophthora* though some species are definitely homothallic others, such as the *P. palmivora* Butler group, may possess definitely homothallic strains, homothallic strains with heterothallic tendencies, heterothallic strains, and neutral or apparently sterile strains. More recently, Helena de Bruyn (**4**) has reported similar complicated conditions in the thallism of *Peronospora parasitica* (Pers.) Tul. De Bruyn inclines to the view, held by Leonian and others, that the heterothallic strains are not strictly unisexual, but possess the potentialities of both sexes, one sex being ordinarily dominant. It is not intended here to discuss the views or evidence on the rôle chemical substances may play in heterothallism in *Phytophthora* (*cf.* **6**, **8**).

The following table gives information on the species studied in the present investigation.

Fungus	Source	No. of single zoöspore cultures	Oöspore formation
Pythium arrhenomanes Drechsl.	J. E. Machacek, Winnipeg. Isolated from wheat roots	39	Sparse
P. Butleri Subramaniam	S. F. Ashby, Imperial Mycological Institute	22	Moderate
P. myriotylum Drechsl.	Centraalbureau voor Schimmelcultures, Baarn	7	Sparse
P. torulosum Coker & Patterson	T. C. Vanterpool. Isolated from wheat roots in England	4	Good
P. complectens Braun	M. Park, Ceylon. Isolated from turmeric (*Curcuma longa* L.)	5	Good
Phytophthora Cactorum L. & C.	S. F. Ashby	9	Good

The method of securing single zoöspore cultures of *Pythium* species now follows. Sterile Syracuse watch glasses, each containing two wheat-root tips in about 3 cc. distilled water, were inoculated with a small piece of cornmeal agar culture of the fungus, and kept at room temperature. After three days the water was changed. This was repeated on the fourth day and the cultures examined thereafter at half-hour intervals for swimming zoöspores. If these were found to be present, a few drops of water containing zoöspores were pipetted off and distributed on the surface of 2 per cent water agar in a Petri dish. If the zoöspores were numerous appropriate dilution in sterile water became necessary. After two hours or more when there was no free water on the agar, the dish was inverted on a microscope stage and a search made for zoöspores separated by several millimetres from the nearest neighbor. As germination of the spores had usually begun by this time, their detection was made easier. The position of each individual spore was marked with a small dot of Indian ink; the dish was then inverted and a disc of agar about 1.5 mm. in diameter cut out from directly above the dot of ink and transferred to another dish containing cornmeal agar. Then followed another microscopic examination to verify that only a single spore had been transferred with each disc of agar. Subsequently, cultures from these single zoöspores were studied separately for the

production of sexual organs and oöspore formation. Cultures of the parent strains were studied concomitantly.

In all the species studied the single spore cultures could not be distinguished from the parent and in every instance developed both oögonia and antheridia, and oöspores, the latter in some species somewhat sparsely, yet no more so than the parent culture. This difficulty of obtaining sexual organs in certain strains of *Pythium* is a common experience. It would appear, however, that sooner or later once the right cultural conditions are provided, sexual organs will form in strains which formerly failed to produce them. The experience of Rands and Dopp (**10**) is a case in point. They found that by using humic acid agar practically all of a large number of recalcitrant strains were induced to develop sexual organs.

From the foregoing evidence, it appears that all of the five species of *Pythium* studied, including the diclinous *P. arrhenomanes,* are homothallic (monoecious, hermaphroditic, self-fertile). The same is true for *Phytophthora cactorum.*

SUMMARY

The complex condition existing in the thallism of certain species of *Phytophthora* and *Peronospora* is pointed out. Strict homothallism is shown to exist in five species of *Pythium* studied. The method used in obtaining the single zoöspore cultures of *Pythium* is described in detail.

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A FEW NEW ZOÖPAGACEAE DESTRUCTIVE TO LARGE SOIL RHIZOPODS

CHARLES DRECHSLER

(WITH 7 FIGURES)

Additional fungi referable to the Zoöpagaceae having been brought to light through inspection of old Petri plate cultures started from various decaying vegetable materials, three of the larger forms among them will be newly described herein. Like most members of the family previously made known, each of the three forms were found subsisting exclusively on a particular species of rhizopod; all other microscopic animals infesting the agar substrata consistently remaining unharmed. The enormous destruction suffered by the protozoan concerned in each instance—destruction amounting often to extermination of all active individuals present—suggests that the feeding operations of the Zoöpagaceae may have an important relation to the pronounced fluctuations in populations of soil protozoa disclosed when determinations of their numbers are made from day to day (**1**).

COCHLONEMA MEGALOSOMUM

The most impressive of the three fungi was found killing off a large *Amoeba* in aging *Pythium* cultures to which had been added some pinches of decaying plant materials collected in open woods with a luxuriant undergrowth of coarse herbaceous weeds, near Beltsville, Md., early in October 1937. As even the smaller infected rhizopods measured about 75 μ in diameter when drawn into an approximately rounded shape (FIG. 1, *A*, *B*), while the larger specimens of similar conformation often attained a width of 125 μ (FIG. 1, *D*), the animals were of dimensions making them visible to the naked eye as minute specks peppered over the surface of the substratum. A thick pellicle surrounded the massive sarcode, whose pseudopodial protuberances it confined usually in broadly lobate contours. One (FIG. 1, *B*, *z*: FIG. 2, *A*, *z*; *B*, *z*),

two (FIG. 2, *C, y, z*) or three (FIG. 1, *D, x, y, z*) digestive vacuoles were often present, and contained always accumulations of somewhat large bacteria. The single large prolate ellipsoidal nucleus, measuring mostly 18 to 24 μ in length and 13 to 19 μ in width, revealed in healthy condition an outer hyaline layer surrounding a perceptibly darker central portion (FIG. 1, *A, n; B, n; C, n; D, n:* FIG. 2, *A, n; B, n; C, n*). Because of a close similarity in nuclear structure thus evident, there can be little doubt that the *Amoeba* concerned here is specifically identical with the one encountered earlier as the prey of a hyphomycete I then described as *Dactylella tylopaga* (**4**). This identity encourages the application again of the binomial *Amoeba verrucosa* Ehrenb., whereby, besides, the rhizopod is distinguished advantageously from the several animals that in accordance with a deplorably indiscriminate usage sanctioned in protozoological writings I have elsewhere discussed as *A. terricola* Greeff. With respect to outward shape and nuclear structure no less than with respect to shape and appearance of contractile and digestive vacuoles, the rhizopod conforms well to at least one of the specimens figured by Leidy as *A. verrucosa* (**8**: *pl. 3, fig. 36*), though differing certainly from some other specimens depicted as likewise illustrative of that species (**8**: *pl. 3, fig. 34, 38*).

Infection of the *Amoeba* is accomplished through germination of an adhering conidium. After the germ-tube has penetrated the pellicle and grown a short distance, usually not much in excess of 5 μ, into the granular sarcode, it gives rise to a terminal expansion (FIG. 1, *A, a*) into which are soon received the entire conidial contents. The globose body thus formed then becomes separated from the germ-tube, and begins autonomous growth within the animal's protoplasmic interior. At the beginning the young thallus (FIG. 1, *A, b:* FIG. 2, *A, a*) shows little to distinguish it from the thalli of other members of the Zoöpagaceae endoparasitic in Amoebae. A more pronounced distal widening than is known in any related form hitherto described becomes evident as the body elongates into the latter half of its first spiral turn (FIG. 1, *A, c:* FIG. 2, *B, a–e*). A maximum width is usually attained when the hypha has made one and one-half turns, a first bifurcation then intervening to reduce the width perceptibly in the two resulting

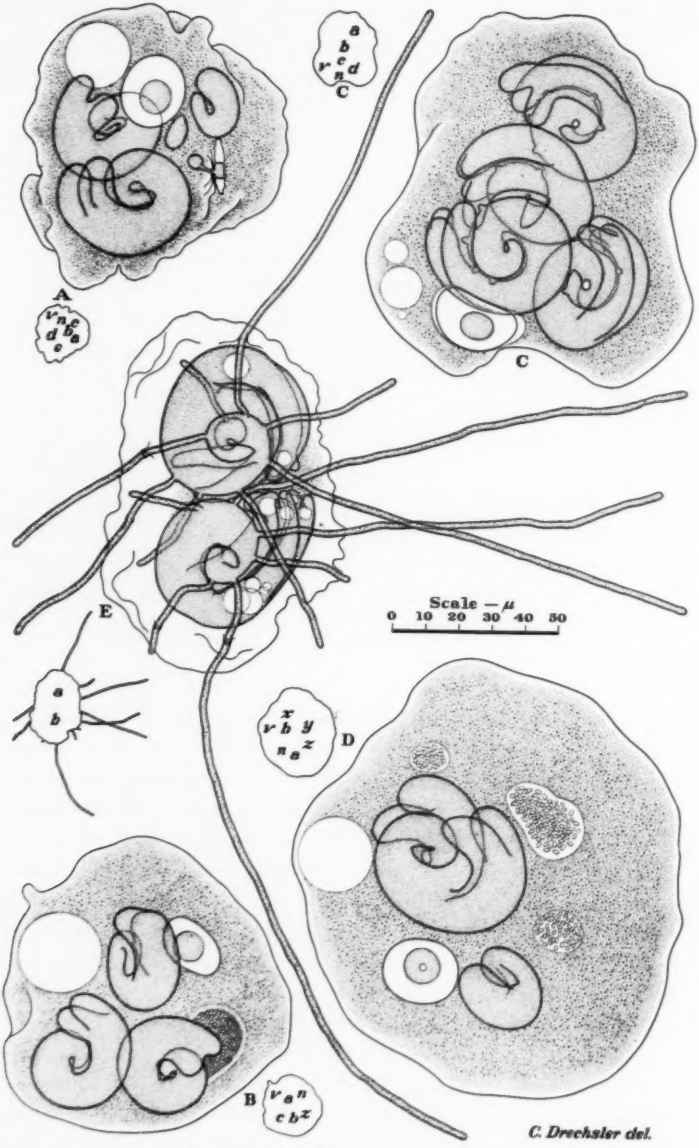

FIG. 1. *Cochlonema megalosomum.*

elements. In instances where a single thallus develops in a large animal, it may describe nearly three successive turns and bifurcate successively four times before the materials of the host are finally exhausted (FIG. 2, *E:* FIG. 3, *A*). Such well developed thalli, more than any other structure now known in the family, reveal the magnificence of form and dimensions that has long made the Zygomycetes a favorite group among mycological virtuosi. Bifurcation here often presents a peculiarity in that it frequently takes place in the plane of the first coil, rather than in a plane perpendicular thereto; so that the daughter elements, instead of being equidistant from and symmetrically placed relative to the axis of the first coil, are represented by an inner branch and an outer one. As a result of this peculiarity in branching, the well-developed thallus of the present species is usually disposed in a markedly flattened spiral rather than in a globose clew as are generally the thalli of *Endocochlus gigas* Drechsl. (**5**) and *Cochlonema megaspirema* Drechsl. (**6**).

The parasite regularly gives rise to profuse conidial apparatus. At a stage when the host animal, if weakened through expropriation in large part of its protoplasmic contents, yet remains capable of movement and continues to operate its contractile vacuole in an apparently normal manner, wart-like protuberances appear on the older or proximal portion of the thallus. Whenever the spiral vegetative body is of relatively small volume, as often when it shares the nourishment in an animal with a number of other similar bodies, these protuberances are restricted closely to the outer profile of the first coil, along which they are usually spaced at approximately equal intervals (FIG. 1, *C, a–d*). Apparently these protuberances undergo little change until the *Amoeba* has been quite disabled from advanced depletion of its substance, when they grow out into filaments (FIG. 2, *D, d, e*) thrusting their way through the pellicle (FIG. 1, *E*) and extending themselves more or less erectly into the air to heights often in excess of 0.5 mm. The aerial hyphae thus formed soon become converted into chains of conidia (FIG. 2, *E*); wherefore the fungus manifestly is to be referred to the genus *Cochlonema.*

Like the homologous filaments of many allied catenulate forms, the continuous aerial hyphae show in their proximal portion little

external modification of any kind, but in their median and distal portions reveal pronouncedly warty sculpturing and regularly spaced constrictions (FIG. 3, *C*). Consequently, the basal members of a conidial chain are mostly cylindrical structures with very short connections, only slightly constricted (FIG. 2, *F:* FIG. 3, *B*, *a–c; E*, *r*), whereas the spores in median and distal positions are shorter, fusiform, pronouncedly warty bodies, tapering toward both bluntly rounded ends (FIG. 2, *G*, *a–z; H*, *a–t:* FIG. 3, *E*, *a–q*, *s–z*), where they are joined to their neighbors by narrow isthmi (FIG. 3, *D*). For the most part the connecting isthmi would seem not wholly empty, since the convex end walls of neighboring conidia often come into contact centrally with one another. In a general way, despite some differences in dimensions and shape, the conidia of the fungus recall those of the congeneric *Cochlonema megaspirema*.

Though large thalli, like the smaller ones, emit conidiiferous hyphae only from their proximal portions, they utilize for such emission a broader expanse of the surface available in these portions (FIG. 2, *E:* FIG. 3, *A*). Regardless of the size of the thallus, the progressive evacuation of protoplasmic contents accompanying production of fertile hyphae was never seen to be associated with the laying down of successive cross-walls so familiar in allied forms. The number and quantity of sporiferous filaments arising from an infected animal depends naturally altogether on the animal's size rather than on the number of thalli participating in its expropriation. Well developed specimens of the susceptible *Amoeba* often yield more than a dozen fertile hyphae (FIG. 3, *A*) that collectively may become visible to the naked eye as a minute whitish speck, and that under a microscope of low magnification appear loosely intertangled in an erect and often impressively luxuriant tuft. Disintegration of the aerial chains leaves hundreds of conidia strewn about on the substratum, each ready to adhere to any susceptible *Amoeba* that may by chance pass over it.

Among several hundred infected animals only three were observed to be instrumental in the development by the parasite of a sexual stage. Judging from this meager material, the fungus would seem to be strictly heterothallic. Thus in each of the seven pairs of sexual hyphae whose conjugation resulted in the seven

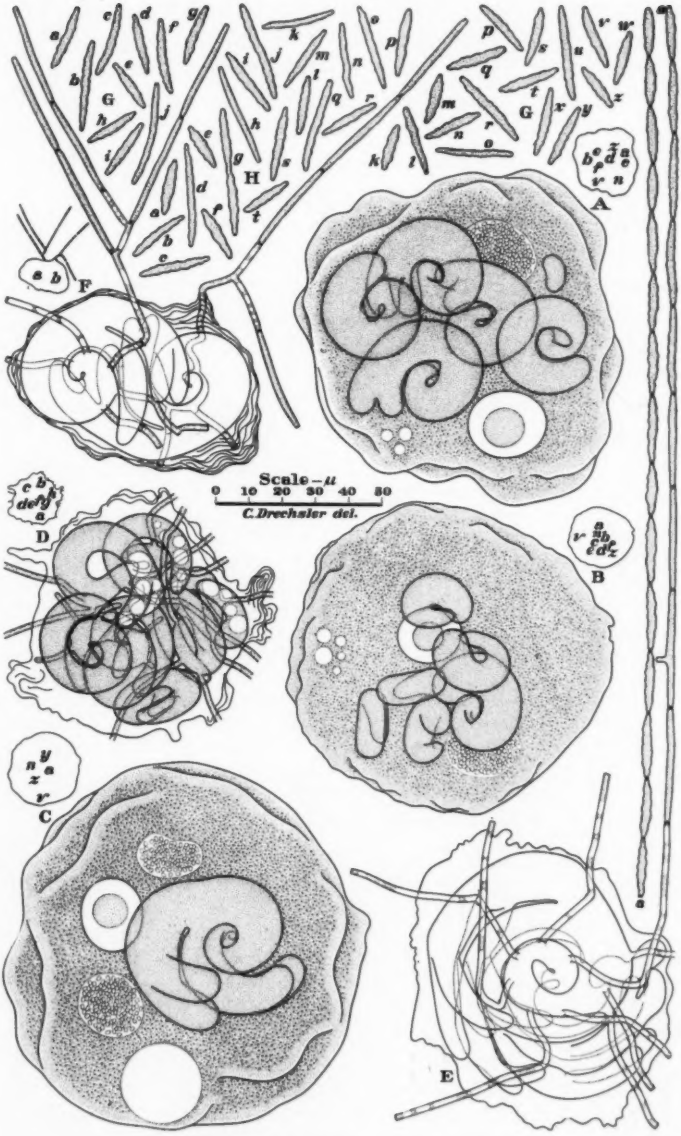

FIG. 2. *Cochlonema megalosomum.*

zygosporangia shown in figure 3, *F*, one hypha has its origin either in thallus *a* or thallus *b*, while the other has its origin either in thallus *c* or thallus *d*. Marked distal inflation of the sexual filaments provides a conspicuous feature in the morphology of the species. The zygosporangia, borne close to the union of the sexual hyphae, are to be reckoned among the largest known in the family. Their stubby protuberances invite comparison more especially with the homologous protuberances of *Cochlonema odontosperma* Drechsl. (**6**), though lacking any cuspidal modification. In two of the thalli that had participated in sexual reproduction, a few heavy crosswalls were observed (FIG. 3, *F, a, d*), these presumably having been inserted as retaining septa at the time the vegetative elements were being evacuated.

A word meaning "large-bodied" is deemed appropriate as a specific name for the fungus.

Cochlonema megalosomum sp. nov.

Hyphae alitae 4–20 μ crassae, semel vel quater repetite dichotomae, semel vel ter spiraliter convolutae, saepe praecipue ubicumque solitariae in animalibus magnis crescentes in spiram mirificam se circumvolventes. Conidia hyalina, 8–38 μ longa, 1.6–3.6 μ crassa, in catenulas 15–35-sporas, plus minusve erectas, 300–700 μ longas digesta, prope basin catenulae fere longiuscula, angusta, levia, cylindrata vel filiformia, in medio et prope apicem catenulae fere latiora, breviora, crasse verrucosa, fusiformia, utrimque abrupte rotundata. Hyphae zygosporiferae 25–75 μ longae, basi circa 1.5 μ crassae, sursum latescentes, apice 6–12 μ crassae, binae ex duabus hyphis alitis enatae. Zygosporangia sphaeroidea, saepe 13–16 μ crassa, 25–45 verrucis circa 1 μ altis basi circa .8 μ latis ornata; zygosporis maturis ignotis.

Amoebam verrucosam (sensu strictiore) enecans habitat in materiis plantarum putrescentibus prope Beltsville, Maryland.

Vegetative hyphae 4 to 20 μ wide, simple or repeatedly dichotomous up to 4 times, and wound spirally into a somewhat flattened coil of 1 to 3 successive turns. Conidia hyaline, 8 to 38 μ (average 20.5 μ) long, 1.6 to 3.6 μ (average 2.6 μ) wide, formed in numbers from 15 to 35 in more or less erect chains often 300 to 700 μ long—those in the proximal portion of a chain usually long, narrow, smooth, cylindrical to filiform—those in the middle and distal portions of a chain shorter, wider, rather coarsely warty, fusiform, abruptly rounded at the ends. Zygophoric hyphae 25 to 75 μ long, approximately 1.5 μ wide at the base, widening to a diameter of 6 to 12 μ at the apex, those of each conjugating pair arising from separate vegetative hyphae, each becoming divided by a

FIG. 3. *Cochlonema megalosomum.*

septum placed 20 to 30 μ below its apex; zygosporangium formed close to the union of the sexual hyphae, subspherical, sessile, usually 13 to 16 μ in diameter, ornamented with 25 to 45 thimble-like protuberances about 1 μ in height and 0.8 μ in basal width; mature zygospores unknown.

Destructive to *Amoeba verrucosa* (*sensu strictiore*) it occurs in decaying herbaceous plant materials in open woods near Beltsville, Md.

In some of the cultures in which *Cochlonema megalosomum* was found multiplying, it encountered competition from two other fungi. One of these fungi, unquestionably a member of the Zoöpagaceae, captured numerous specimens of *Amoeba verrucosa* by adhesion to sparse continuous mycelial filaments mostly about 2 μ wide, and intruded into each captive an extensively branched, bushy haustorium most similar to the haustorium of *Zoöpage mitospora* Drechsl. (**7**). In many instances the fungus captured animals already infected by *C. megalosomum;* both of the organisms unconcernedly preceeding with their normal development side by side, and in apparently congenial relationship dividing the protoplasmic materials of the rhizopods between them.

The other of the two fungi competing with *Cochlonema megalosomum* has likewise become known only by its vegetative stage, which is, however, of truly remarkable character, consisting of narrow filamentous hyphae that singly pursue conspicuously straightforward courses on the surface of the substratum for distances varying mostly between 0.5 mm. and 5 mm. before bifurcating abruptly and symmetrically at angles of approximately 120 degrees. Each of the resulting elements repeats the prolonged straightforward course and geometrical bifurcation of its parent, with the result that an exceedingly sparse arachnoid mycelial network is soon extended far over the substratum. Any specimen of *Amoeba verrucosa* captured on a filament of the predacious zoöpagaceous form in close proximity to one of the conspicuously straightforward hyphae, is promptly invaded by a branch from that hypha, and depleted of its protoplasmic material by a copious, somewhat inflated, apparently septate, ramifying haustorial system arising from the branch. Such haustorial elements as may have been intruded into the animal by the zoöpagaceous form are caused to

degenerate completely, the newcomer thus excluding the true captor from further utilization of its prey. Now and then, also, a specimen of *A. verrucosa* disabled by *C. megalosomum* was found invaded by a branch from the arachnoid fungus, but as disablement of an animal by the endoparasite usually does not occur until the fleshy contents have been largely depleted, the intruder here developed only meager haustorial apparatus which exerted no noticeable ill effects on the massive spiral thalli nearby.

COCHLONEMA BACTROSPORUM

Among the larger testaceous rhizopods that multiply in old agar plate cultures following the addition of decaying herbaceous vegetable detritus, is a member of the genus *Heleopera* Leidy agreeing well with the description of *H. sylvatica* Penard (**9**: p. 389–390), and accordingly referred to that species. After a few weeks of development under favorable conditions, the animal often attains populations in excess of 100 individuals. In a number of cases such flourishing colonies of the rhizopod then suffered a rapid reduction of living specimens that apparently always ended in complete extermination.

That the agent responsible for the devastation might most probably be a parasite belonging to the Zoöpagaceae was immediately suggested by the presence of more or less erect, isolated tufts, each composed of 2 to 10 tall aerial filaments arising from the substratum in immediate proximity to one of the animals (FIG. 4, *A*). On closer examination the aerial filaments were indeed revealed as consisting either of chains of rod-like conidia (FIG. 4, *B, a, c, d, e:* FIG. 5, *A, a–c*) or of continuous hyphae manifestly representing younger stages in the development of such chains (FIG. 4, *B, b*). While the individual conidia rather strongly resembled those of *Cochlonema cylindricum* Drechsl. (**6**) in shape, they offered easily recognizable differences not only in their greater dimensions, but also in the persistance of a somewhat papillate protrusion at each of their ends. These curious polar modifications have their origin in the partial delimitation of the conidia from one another through localized evacuation of protoplasm in such a manner as to leave an empty groove surrounding a narrow axial isthmus; separation of

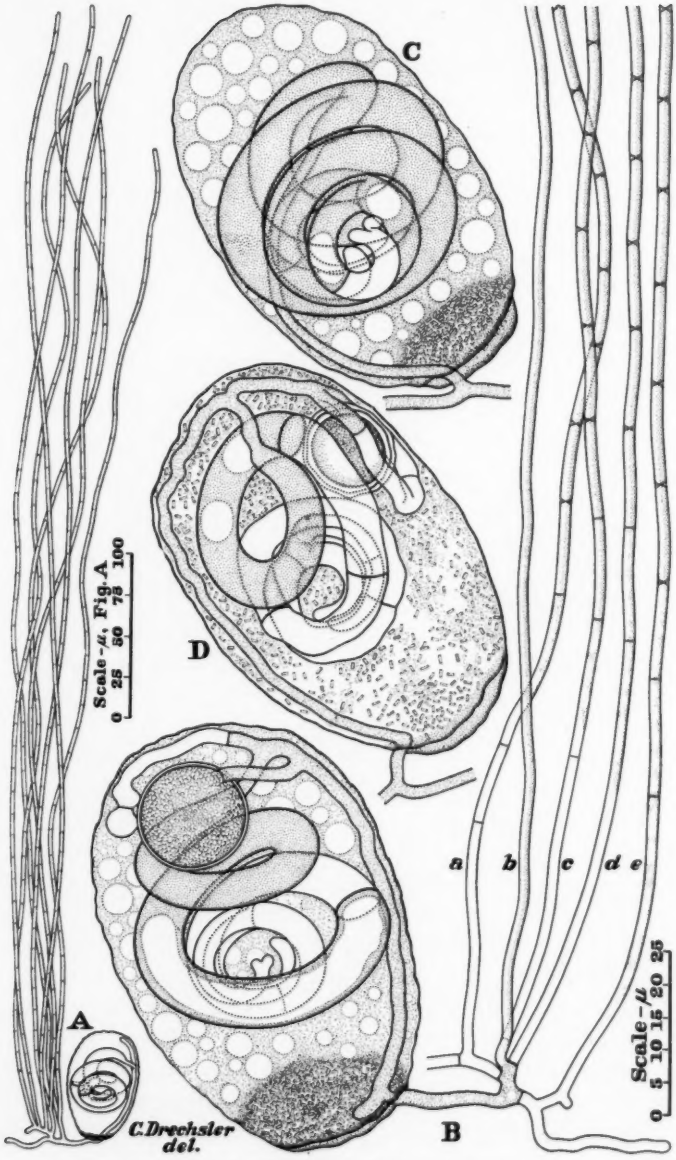

FIG. 4. *Cochlonema bactrosporum.*

adjacent spores being then completed by the insertion of a cross-wall at the middle of the isthmus. It appears possible that conidial development may follow an essentially similar course in *C. cylindricum,* even if the spores of that form show little distinctive outward modification.

As was expected from the character of the conidial apparatus that they represented, the tufts of aerial filaments were in each instance found to have a close hyphal connection with a spiral thallus inside the specimen of *Heleopera sylvatica* nearby (FIG. 4, *A, B:* FIG. 5, *A*). A few thalli that could be followed satisfactorily under the difficult optical conditions brought about by the murky, vacuolate consistency of the surrounding protoplasm, showed very handsome helicoid arrangement: the vegetative hypha in its first turn, usually nearest the fundus of the host, widening gradually to a maximum diameter maintained throughout the grandiose second turn, then gradually diminishing in width in the narrowing distal turns closest to the mouth of the animal (FIG. 4, *B, C*). Branching was observed only in the distal portion of the thallus, a first bifurcation often occurring at the beginning of the fourth turn, and a second, where present, at the beginning of the fifth turn. The meager and inconspicuous branching is hardly sufficient to counteract the deceptive impression that the vegetative body usually conveys of having grown opposite to its actual direction of growth. After the initiation of reproductive development, the thallus is progressively evacuated of protoplasm from the distal toward the proximal portion, and retaining septa are laid down in positions successively closer to the proximal end (FIG. 4, *A–D*). The single reproductive filament arising from the proximal end of the thallus often needs to thrust its way almost the entire length of the animal before it can emerge from the oral opening. Once outside of its host, the reproductive filament sends a few short branches into the substratum, and then gives rise successively to the aerial hyphae that, except for a sterile basal part, become converted, likewise successively, to chains of cylindrical conidia.

Aside from its copious display of conidial apparatus, the fungus also reproduces sexually. Approximately a third of the animals seen destroyed were partly utilized in the development of zygospores. The sexual spores of the parasite, unlike any of the

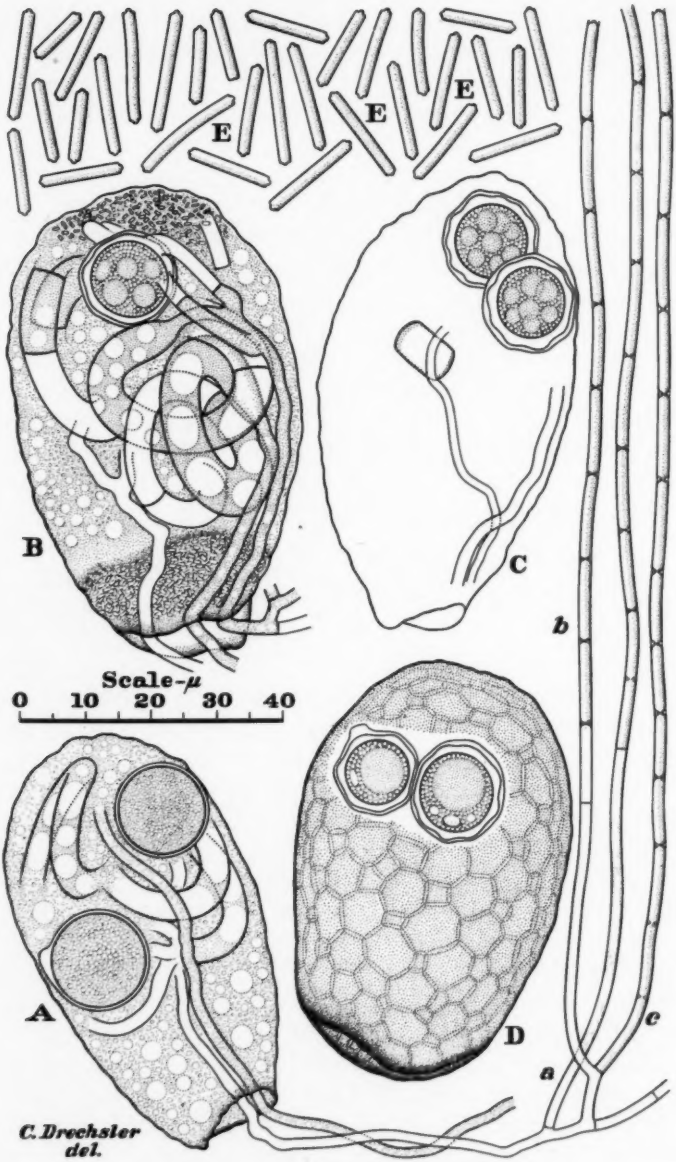

FIG. 5. *Cochlonema bactrosporum.*

homologous bodies hitherto made known in related species, usually are formed within the host—more often indeed, in positions near the fundus than near the mouth, and only rarely in the substratum a short distance from the mouth. As frequently an animal in which a zygospore was visible contained only a single thallus, it would seem that the fungus is definitely homothallic (FIG. 4, *B, D*). Apparently the zygosporangium is formed at the union of two rather short sexual elements, which in some cases (FIG. 4, *B*) arise separately from the reproductive filament short distances from its origin, and in other cases arise through bifurcation of a single branch similarly given off by the reproductive filament close to its attachment (FIG. 4, *D*). On attaining its definitive size the zygosporangium consists of a smoothly spherical cell, densely filled with coarsely granular contents (FIG. 4, *B:* FIG. 5, *A*). The coarsely granular protoplasmic structure is retained during the earlier stages in the development of the zygospore proper, when there is deposited within the zygosporangial wall a thicker wall having perceptible sigillate thickenings. These thickenings become slightly more pronounced as maturation proceeds with accompanying formation and enlargement of reserve vacuoles, often from 5 to 10 in number (FIG. 5, *B, C*). These vacuoles later coalesce, so that in its fully mature condition the zygospore of the fungus under consideration, like that of other members of the Zoöpagaceae, shows an internal organization more familiar in oöspores: a large central reserve globule being surrounded by a parietal protoplasmic layer of coarsely granular texture, in which is imbedded on one side an oblate ellipsoidal refringent body (FIG. 5, *D*).

As in all instances where an animal clearly was parasitized by a single thallus, only a single reproductive filament could be seen emerging from the oral opening and no more than one zygospore could be discerned developing inside. It is believed that plural production of these structures from individual thalli, if occurring at all, is at least infrequent. The presence of two or three reproductive filaments (FIG. 5, *A, B, C*), or of two zygosporangia (FIG. 5, *A, C, D*) would therefore, in the main, seem to imply the presence of a corresponding number of thalli. Plural infection would appear to be concerned also in cases where spiral hyphal coils, only

partly visible, are found in markedly confused arrangement (FIG. 5, *A, B*).

A term made up from words meaning "staff" and "seed," respectively, and intended to be descriptive of the cylindrical conidium, is deemed appropriate as a specific name for the fungus.

Cochlonema bactrosporum sp. nov.

Hyphae alitae 2–8 μ latae, primo latescentes deinde minuentes, ter vel quater in spiram primo latescentem deinde minuentem cocleatim convolutae, saepius semel vel bis sursum dichotomae, unusquisque ex basi hypham filiformem 1.5–2.5 crassam evolvens quae non solum in aera extra testam animalis invasi 2–10 hyphas erectas 300–750 μ altas deinceps profert sed subinde etiam binos ramos zygosporiferos intra testam emittit; hyphis aeriis se vertentibus in conidia catenulata, hyalina, levia, cylindrata, 9–19 μ longa, 1.6–1.9 μ crassa, utrimque ad instar verruculae rotundata; ramis zygosporiferis 10–45 μ longis, circa 2 μ crassis; zygosporangiis sphaeroideis 13–17 μ crassis, membrana postea circum zygosporam collapsa; zygosporis paulo flavidis, plerumque 11–15 μ crassis, maturitate ab membrana 0.5–1.8 μ crassa in extremitate ad instar sigillorum undulata circumseptis.

Heleoperam sylvaticam enecans, habitat in reliquiis plantarum putrescentibus prope Beltsville, Maryland.

Vegetative hypha disposed in a more or less extended helicoid spiral of 3 or 4 successive turns; attaining a width of 5 to 8 μ in the second and widest turn that often measures 35 to 40 μ across; in the successively smaller third and fourth turns often narrowing to a diameter of 2 or 3 μ; sometimes branching dichotomously once or twice in its distal portion; putting forth from its base a filament 1.5 to 2.5 μ wide that not only produces successively 2 to 10 erect aerial hyphae 300 to 750 μ high outside the testa of the host animal, but in addition sometimes gives rise, most often inside the testa, to paired zygophoric branches: each of the aerial hyphae becoming converted into a chain of hyaline conidia 9 to 19 μ (average 14.3 μ) long, 1.6 to 1.9 μ wide, smooth, cylindrical, with a small wart-like protuberance at both ends; the zygophoric hyphae, 10 to 45 μ long and about 2 μ wide, after conjugation bearing a subspherical zygosporangium 13 to 17 μ in diameter and surrounded by a wall originally smooth but later collapsing rather closely about the yellowish zygospore, which measures usually 11 to 15 μ in diameter and at maturity is surrounded by a wall 0.5 to 1.8 μ thick with sigillate undulating outer contour.

Destroying *Heleopera sylvatica* it occurs in decaying plant remains on moist ground near Beltsville, Md.

ACAULOPAGE MARANTICA

One of the old maize-meal agar plate cultures, in which *Cochlonema megalosomum* made its appearance, showed also the development of another relatively coarse though morphologically less remarkable member of the Zoöpagaceae. This second fungus subsisted exclusively on a large *Amoeba* that often attained a length of 150 μ when drawn into its usual slightly elongated form (FIG. 6, *A, B, C*). The animal was further distinguished by a thickish pellicle cast in boldly undulating contours over broad pseudopodial protrusions, as well as by a single large prolate ellipsoidal nucleus often 30 to 35 μ long and 15 to 20 μ wide, the generally homogeneous consistency of which was interrupted close under the delimiting membrane by a parietal layer of noticeably darker material, appearing in profile as disjunctive rods arranged end to end. From these morphological features the identity of the rhizopod with the one previously found serving *Endocochlus gigas* as prey and *C. megaspirema* as host was sufficiently evident; wherefore it will be referred to as *Amoeba terricola* Greeff (*sensu strictiore*).

Nourished on an abundant supply of living shelled rhizopods, including mainly *Trinema enchelys* Ehrenb. (FIG. 6, *B*) and a small species of *Euglypha* (FIG. 6, *A–C*), the large *Amoeba* had multiplied until more than 100 individuals were present. The animals, which because of their size were clearly visible to the naked eye, first invited attention through an abnormally dense concentration of some dozens of specimens in a small area adjacent to a pinch of decaying herbaceous material planted there some weeks earlier—the crowded tract presenting a rather densely stippled macroscopic appearance. Examination under the microscope revealed a relationship wholly analogous to that described earlier (2) as existent between the large but decidedly different *Amoeba* then provisionally designated as *A. terricola* III, on the one hand, and the predacious *Zoöpage planera* Drechsl. on the other. At the margin of the crowded area specimens of *A. terricola* (*sensu strictiore*) were seen adhering to hyphae of an aseptate, irregularly disposed mycelium, some of the hyphae, indeed, being enveloped here and there in pseudopodial folds (FIG. 1, *A*). The small number of stalked, dichotomously branched haustoria thrust into the individ-

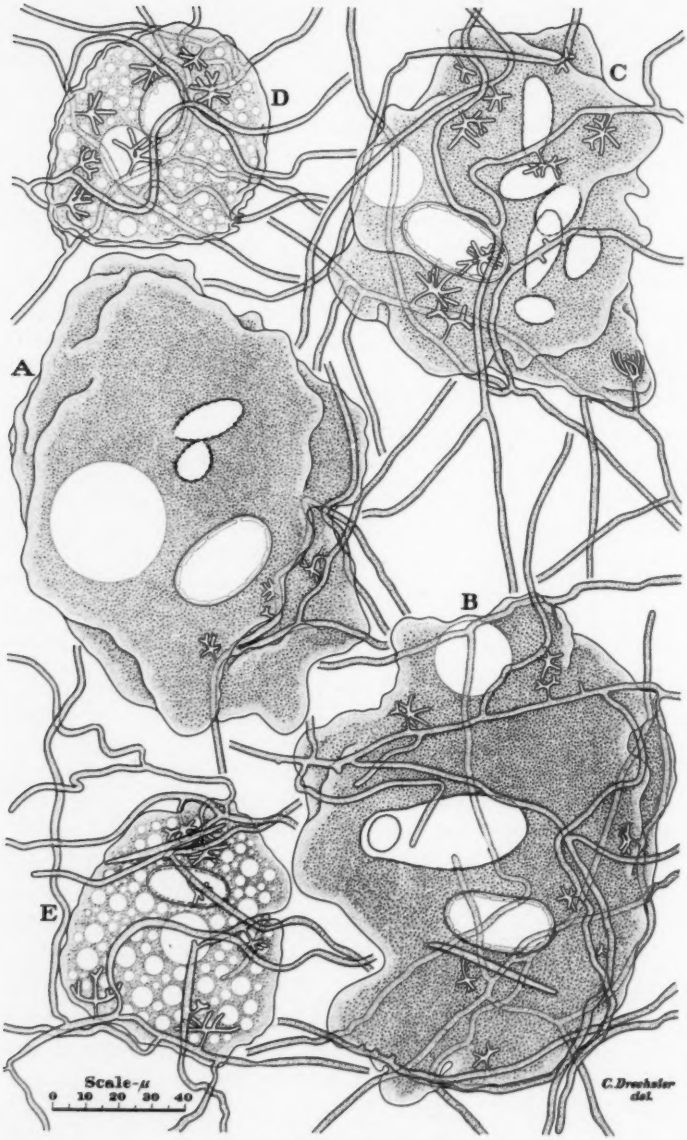

FIG. 6. *Acaulopage marantica.*

ual animal, together with the apparently undiminished density of the granular protoplasm, the unceasing extension and retraction of pseudopodia in attempted locomotion, the normal discharge of the contractile vacuole and the healthy appearance of the large nucleus, gave evidence that expropriation of the rhizopod was still in an early stage. By far the larger number of closely congregated animals showed much more extensive enwrapment by the mycelium (FIG. 6, *B, C*). A conspicuously irregular disposition of the enveloping branched filaments, and their presence not only on the under side of each captive but on its upper exposed side as well were to be explained undoubtedly by the persistent wallowing movements through which the animal, instead of freeing itself, merely succeeded in enmeshing itself more securely. Intrusion of additional haustoria consequent to more extensive mycelial engagement hastened the depletion of the captive's fleshy contents: the diminishing cytoplasm becoming pronouncedly vacuolate (FIG. 6, *D*); the large nucleus betraying internal degeneration in a gradual obliteration of its interrupted darker layer (FIG. 6, *E*). Eventually nothing remained visible but the empty collapsed pellicle, within which the haustoria had disappeared from view when their protoplasmic contents had been withdrawn into the parent mycelial filaments.

The fungus reproduced asexually by giving rise to an abundance of elongated fusiform conidia, appendaged at both ends. Development of the individual conidium was found to begin with the production from a superficial hypha of an erect continuous outgrowth narrowing for a short distance above its base, then rather markedly widening upward for a somewhat greater distance, and then again gradually narrowing throughout the distal two-thirds of its length (FIG. 7, *A; B, c*). From the slender apical portion embracing about a third of the length of the outgrowth the contents were then retracted, and a confining septum was deposited to delimit the filled from the empty part. In the meantime a cross-wall had been inserted in the narrow isthmus a short distance above the base of the outgrowth, a small segment immediately above the cross-wall being thereupon emptied of protoplasm, and another confining septum deposited (FIG. 7, *B, a, b, d–g*). Through disarticulation at the lowest septum, the conidium with its short empty

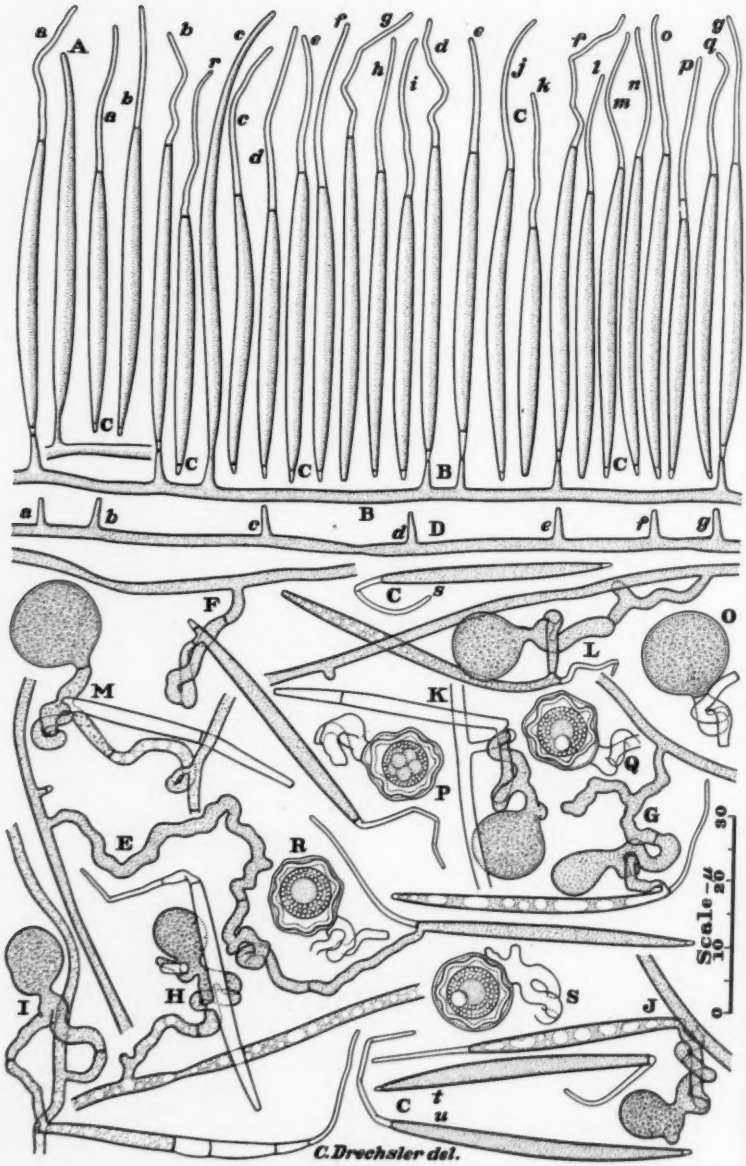

FIG. 7. *Acaulopage marantica.*

basal stipe, its spindle-shaped living cell, and its more or less collapsed, narrow, empty distal appendage (FIG. 7, *C, a–f, h–j, m–u*) became separated from the short erect sterigma. Except for its considerably greater dimensions, and for the presence of its empty basal stipe, which, for that matter, was occasionally absent (FIG. 7, *C, g, k, l*), the conidium strongly resembles that of the obviously congeneric *Acaulopage rhicnospora* Drechsl. (**3**), though the close bristling arrangement of the erect spores on the prostrate filaments would seem more especially suggestive of *A. cercospora* Drechsl. (**5**).

Sexual reproduction of the fungus took place abundantly in the culture, and as in many related forms appeared to be further encouraged when portions of occupied substratum were removed to glass slides for microscopic examination. Of each pair of zygophoric branches, one always came from a mycelial filament, while the other consisted of a germ-tube from a conidium. Instances of conjugation between two branches arising from mycelial hyphae, or of conjugation between two germ-tubes were never seen among the hundreds of units of sexual apparatus that came under observation. Contact of the zygophoric branches was found associated usually with only slight twisting, and was regularly accompanied by the deposition of cross-walls partitioning off the paired gametangia (FIG. 7, *E, F*). The subspherical zygosporangium was developed generally on a rather short stalk arising at a variable but usually inconsiderable distance from the union of the sexual hyphae (FIG. 7, *E–M*). Delimitation of the organ by a basal septum (FIG. 7, *M, O*), and conversion of the contents into a zygospore with a sculptured wall of undulating profile well separated from the sporangial envelope except at the projecting crests, followed a sequence familiar in most other members of the family (FIG. 7, *P–S*). In its mature condition the zygospore showed a relationship of parts hitherto found only in species of *Endocochlus* —the inner surface of the thick undulate wall appearing distinctly separated from the subspherical protoplast (FIG. 7, *P–S*). The protoplast, as in related forms, revealed, on full maturation, a single central reserve globule surrounded by a parietal layer of uniform granules; and imbedded in the granular layer was usually discernible a globular refringent body (FIG. 7, *Q–S*).

Though the fungus was active in a culture that permitted abundant development also of *Cochlonema megalosomum,* it was never observed to capture specimens of *Amoeba verrucosa,* nor, on the other hand, was *C. megalosomum* observed to parasitize *A. terricola* (*sensu strictiore*). Like the endoparasite, however, and, indeed, to a greater extent and more frequently, it suffered from interference by the sparsely arachnoid, geometrically disposed mycelial form of intrusive propensities. The arachnoid form by itself was evidently no more able to attack *A. terricola* than *A. verrucosa,* but when once a specimen of the former was captured, it extended within the captive its characteristically swollen, branching haustorial system, the presence of which promptly caused degeneration in the haustoria of the predacious species under consideration, just as it brought about degeneration in the haustoria of the unindentified predacious form often destructive to *A. verrucosa.*

A term meaning "withered" and believed to be aptly descriptive of the conidia of the fungus, with their frequently collapsed distal appendages, is offered as an appropriate specific name.

Acaulopage marantica sp. nov.

Mycelium ramosum, effusum; hyphis hyalinis, saepe irregulariter flexuosis, 1–2.8 μ crassis, ad animalcula inhaerentibus, vulgo ea extense circumplicantibus, pelliculam eorum perforantibus, haustoria intus evolventibus quae carnem exhauriunt; haustoriis pedicellatis, pedicello saepius 2.5–6 μ longo, 0.6–1 μ crasso, apice abrupte latescente, semel vel ter repetite bifurco, ita 2–8 ramulos divaricatos, 1–10 μ longos, 0.8–1.6 μ crassos ferente. Conidia hyalina, ex sterigmatis erectis, vulgo 3–5 μ altis, basi 1–1.5 μ crassis, sursum attenuatis, apice 0.5–0.8 μ crassis, inter se saepius 5–35 μ distantibus oriunda, vulgo ex partibus tribus composita: parte supera vacua, 15–30 μ longa, basi 0.8–1.3 μ crassa, sursum leniter attenuata, saepius plus minusve marcida vel collapsa; parte media protoplasmatis repleta, elongato-fusoidea, 33–52 μ longa, 2.4–3.1 μ crassa; parte infera vacua, 0.8–4 μ longa, sursum 0.6–1.2 μ crassa. Hyphae zygosporiferae 10–75 μ longae, 1–3 μ crassae, saepe plus minusve irregulariter flexuosae, 10–15 μ ab junctione septo divisae, interdum aliquantulum inter se circumplicantes, una ex hypha mycelii, altera ex conidio germinanti oriunda. Zygosporangia primo levia, sphaeroidea, vulgo 12–14 μ crassa, maturitate membrana circa zygosporam laxe collapsa; zygospora flavida, globosa, circa 9–12 μ crassa, maturitate membrana late verrucosa vel sinuosa, cellulam viventem sphaeralem 6.5–8 μ latam laxe circumdante.

Amoebam terricolam (sensu strictiore) capiens consumensque habitat in materiis plantarum putrescentibus prope Beltsville, Maryland.

Mycelium branched, spreading; vegetative hyphae colorless, often irregularly flexuous, 1 to 2.8 μ (mostly about 2 μ wide), ad-

hering to and often becoming extensively wrapped about minute animals, penetrating the pellicle of each captive and intruding haustoria to appropriate the fleshy contents; haustoria pedicellate, the pedicel often 2.5 to 6 μ long, 0.6 to 1 μ wide, abruptly widening and bifurcating one to three times at wide angles to terminate in 2 to 8 branches 1 to 10 μ long and 0.8 to 1.6 μ wide. Sterigmata arising abruptly from superficial hyphae at intervals of 5 to 35 μ, erect, 3 to 5 μ high, 1 to 1.5 μ wide at the base, tapering upward to a width of 0.5 to 0.8 μ at the tip whereon is borne erectly a single conidium. Conidia hyaline, each usually composed of three parts: the distal part empty, 15 to 30 μ (average 21.6 μ) long, 0.8 to 1.3 μ (average 1.1 μ) wide at the base, tapering upward slightly, often more or less collapsed; the middle part filled with protoplasm, elongate spindle-shaped, 33 to 52 μ (average 42.9 μ) long, 2.4 to 3.1 μ (average 2.7 μ) wide; the lowest part obconical, empty, 0.8 to 4 μ (average 1.5 μ) long, 0.6 to 1.2 μ wide at its distal attachment. Zygophoric hyphae 10 to 75 μ long, 1 to 3 μ (mostly about 2 μ) wide, often more or less irregularly flexuous, the two of a pair sometimes slightly intertwined, one commonly arising as a branch from a mycelial filament, the other commonly consisting of a germ-tube from a conidium, each with a distal portion, or gametangium, 10 to 15 μ long, set off by a septum. Zygosporangium at first smoothly subspherical and commonly 12 to 14 μ in diameter, at maturity collapsing loosely about the zygospore; the latter yellowish, 9 to 12 μ in diameter, its wall, at maturity broadly verrucose and pronouncedly sinuous in contour, loosely surrounding a subspherical living cell usually 6.5 to 8 μ in diameter.

Capturing and consuming *Amoeba terricola* (*sensu strictiore*) it occurs in decaying plant remains near Beltsville, Md.

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EXPLANATION OF FIGURES

FIG. 1. *Cochlonema megalosomum;* drawn with the aid of a camera lucida to a uniform magnification; × 500 throughout. *A*, specimen of *Amoeba verrucosa* in a condition of active motion, showing besides its nucleus, *n*, and its contractile vacuole, *v*, five thalli of the fungus, *a–e: a* representing a very early stage, protoplasmic materials still being received from the externally adhering conidium; *b*, a stage soon after separation from the parent conidium; *c*, a thallus of one turn with marked distal widening; *d* and *e*, older and more massive thalli, each of which has undergone a single bifurcation. *B*, specimen of *A. verrucosa* in active state, containing three bifurcating thalli of the fungus, *a–c*, besides its own nucleus, *n*, its contractile vacuole, *v*, and a digestive vacuole, *z*, inclosing many ingested bacteria. *C*, specimen of *A. verrucosa* containing besides its nucleus, *n*, and its contractile vacuole, *v*, four thalli of the fungus, *a–d*, of which two, *b* and *d*, are twice dichotomous throughout, the others, *a* and *c*, showing a second dichotomy in only one of the two elements resulting from the first bifurcation; in each of the thalli hyphae are just beginning to grow out from the outer contour of the older or proximal portion. *D*, a large specimen of *A. verrucosa* in active state, showing a rather young thallus, *a*, and an older, larger, successively twice bifurcate thallus, *b*, of approximately two successive turns, besides revealing its own nucleus, *n*, its contractile vacuole, *v*, and three digestive vacuoles, *x–z*, surrounding groups of ingested bacteria. *E*, pellicle of *A. verrucosa*, the contents of which have been depleted by two thalli, *a* and *b*, that have put forth, respectively, eight and six sporiferous hyphae of variable lengths.

FIG. 2. *Cochlonema megalosomum;* drawn with the aid of a camera lucida to a uniform magnification; × 500 throughout. *A*, specimen of *Amoeba verrucosa* in active condition, infected with a very young thallus, *a*, and five much larger thalli, *b–f; n*, nucleus of host animal; *v*, contractile vacuole in incipient stage of expansion; *z*, digestive vacuole containing numerous ingested bacteria. *B*, specimen of *A. verrucosa* in state of active locomotion, containing six thalli, *a–f; n*, nucleus of host animal; *v*, contractile vacuole in incipient stage of expansion; *z*, digestive vacuole containing numerous ingested bacteria. *C*, specimen of *A. verrucosa* in a state of active locomotion, though burdened with the large thallus *a; n*, nucleus of host animal; *v*, contractile vacuole; *y* and *z*, digestive vacuoles containing numerous ingested bacteria. *D*, shrunken pellicle of *A. verrucosa*, the protoplasmic contents of which have been consumed by the eight thalli, *a–h;* from

seven of the thalli, *b–h*, sporiferous hyphae have begun to grow out. *E*, wrinkled pellicle of *A. verrucosa* surrounding an empty envelope of a large spiral thallus whose protoplasmic contents have been used up in the production of eight conidiiferous filaments; of these filaments seven are represented, from lack of space, only by their sterile basal parts, the other one being shown in two sections connecting at the common point *a*. *F*, wrinkled pellicle of *A. verrucosa* surrounding two largish empty envelopes of thalli that have been exhausted of contents in the production of conidiiferous hyphae, whereof, from lack of space, only proximal portions are shown. *G*, *a–z*; *H*, *a–t*, conidia showing variations in dimensions, shape and sculpturing.

FIG. 3. *Cochlonema megalosomum;* drawn with the aid of a camera lucida to a uniform magnification; × 1000 throughout. *A*, collapsed pellicle of *Amoeba verrucosa*, the fleshy contents of which have been consumed in the growth of an unusually large thallus, now represented only by an empty aseptate envelope, owing to the depletion of its protoplasm in the production of eighteen conidiiferous hyphae; from lack of space only the proximal portions of the fertile hyphae are shown. *B*, a conidiiferous filament branching within the host pellicle, and showing long, smooth conidia in the proximal portions of spore chains. *C*, distal portion of growing aseptate conidial filament, showing regularly spaced constrictions and coarsely warty sculpturing. *D*, distal portion of mature conidial chain, showing narrow contact of adjacent conidia at the axis of each isthmus. *E*, conidia, showing variations in dimensions, shape and sculpturing. *F*, collapsed pellicle of *A. verrucosa* within which are found four empty thalli of the parasite, *a–d;* from these thalli have been produced zygophoric hyphae that have united in pairs by their greatly widened apices to give rise to the handsomely mammillated zygosporangia *e–k;* as the two zygosporangia *e* and *k* have each resulted from union of paired hyphae arising from thalli *a* and *c*, the two zygosporangia *f* and *i* each from union of paired hyphae arising from thalli *b* and *d*, and the three zygosporangia *g*, *h* and *j* each from union of paired hyphae arising from thalli *a* and *d*, presumably the thalli *a* and *b* are of one sexual constitution, and the thalli *c* and *d* of the opposite sexual constitution.

FIG. 4. *Cochlonema bactrosporum;* drawn with the aid of a camera lucida to a uniform magnification. *A*, depleted specimen of *Heleopera sylvatica* containing the empty envelope of a single thallus that has used up its contents in the production of a tuft of seven conidial chains; × 250. *B*, specimen of *H. sylvatica* containing a thallus that has given rise inside of the testa to a young but fully grown zygosporangium, and outside of the testa to a tuft of five erect asexual reproductive filaments, *a–e*, of which, for lack of space, only the proximal portions are shown—while the continuous filament *b* is in a young growing condition, the others, *a*, *c*, *d* and *e*, consist largely of conidial chains; × 1000. *C*, specimen of *H. sylvatica* containing a well developed thallus of the parasite, from whose younger branched extremity protoplasm is being withdrawn for the extension of conidial filaments outside of the testa; × 1000. *D*, specimen of *H. sylvatica* containing a thallus from which the protoplasmic contents have mostly been withdrawn

to provide for the formation within the animal's testa of a zygospore, and of conidiiferous filaments outside of the testa; × 1000.

FIG. 5. *Cochloncma bactrosporum;* drawn with the aid of a camera lucida to a uniform magnification; × 1000 throughout. *A,* specimen of *Heleopera sylvatica* within which are indistinctly visible several curving portions of hyphae belonging probably to two separate thalli; two zygosporangia of definitive size, but still immature, are more clearly visible inside, as are also two hyphae growing out of the animal's mouth, one of which has given rise to three conidial chains, *a–c,* whereof, from lack of space, only basal portions are shown. *B,* specimen of *H. sylvatica* containing presumably three thalli of the parasite, each extending a separate filament through the oral opening to produce conidial chains externally; one having, in addition, produced a zygosporangium with nearly mature zygospore, inside of the testa. *C,* depleted specimen of *H. sylvatica* within which a small segment of thallus, two outgrowing filaments, and two zygosporangia with nearly mature zygospores are visible. *D,* depleted specimen of *H. sylvatica,* showing inside of the testa with its characteristic arrangement of plates, two fully mature zygospores, each surrounded by its sporangial membrane. *E,* conidia, showing variations in dimensions and shape.

FIG. 6. *Acaulopage marantica;* drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A,* specimen of *Amoeba terricola (sensu strictiore)* in active condition, entangled on one side with adhesive mycelial filaments, from which four haustoria have been thrust through the pellicle into the sarcode; within the animal are shown also the fully expanded contractile vacuole, the large nucleus, and two depleted testae of ingested rhizopods referable apparently to the genus *Euglypha. B,* living specimen of *A. terricola* extensively inwrapped in adhesive mycelial filaments, from which collectively eight haustoria have been thrust into the sarcode exclusive of the haustorium arising from an adhering conidium; within the animal's body are shown also its contractile vacuole, its ellipsoidal nucleus, and the large empty testa of an ingested specimen of *Trinema enchelys. C,* a living specimen of *A. terricola* extensively inwrapped with adhesive mycelial filaments from which collectively ten haustoria have been thrust into the sarcode; within the animal are distinguishable its contractile vacuole, its ellipsoidal nucleus, and six empty testae of ingested rhizopods belonging apparently to a small species of *Euglypha. D,* a rather small specimen of *A. terricola* securely entangled in adhesive mycelial filaments; the abundance of vacuoles distributed through the sarcode indicating a somewhat advanced stage of protoplasmic depletion, accomplished by means of the seven well developed haustoria. *E,* another smallish specimen of *A. terricola* entangled in adhesive mycelial filaments; from these filaments and a germinating conidium have been intruded eight haustoria, which have brought about a well advanced stage of protoplasmic depletion in the animal, as is manifest from the presence of very numerous vacuoles, and from the partial obliteration of the interrupted parietal layer in the nucleus.

FIG. 7. *Acaulopage marantica;* drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A,* portion of prostrate

hypha on which is borne an erect process later to develop into a conidium. *B*, a prostrate filament with seven conidia, *a–g*, borne erectly on separate erect sterigmata; all of the conidia are mature except one, *c*, which though of approximately definitive size, has not been partitioned off from its sterigma, nor undergone evacuation either in its proximal or in its distal part. *C*, *a–u*, conidia, showing variations in dimensions and shape of living cell, empty basal stipe, and empty distal appendage. *D*, prostrate hypha with seven erect sterigmata, *a–g*, all denuded of their conidia. *E*, *F*, young sexual apparatus; in each unit a zygophoric hypha arising as a branch from a mycelial filament has fused apically with another zygophoric hypha arising as a germ-tube from a fallen conidium. *G–L*, sexual apparatus in somewhat later stages of development, likewise showing origin of each unit through union of one zygophoric hypha, arising as a branch from a mycelial filament, with another consisting of a germ-tube from a conidium. *M*, sexual apparatus at still later stage, showing the fully grown zygosporangium set off from its supporting stalk by a septum. *O*, fully grown zygosporangium set off by a septum from evacuated zygophoric elements. *P*, nearly mature zygospore lying within the somewhat collapsed sporangial membrane, to which is shown attached the empty envelope of the united gametangia. *Q–S*, mature zygospores, each lying within its slightly relaxed sporangial envelope.

EMPUSA INFECTIONS OF THE HOUSE-FLY IN RELATION TO MOISTURE CONDITIONS OF NORTHERN IDAHO

CHARLES C. YEAGER

The common fungus, *Empusa Muscae,* infecting house-flies is a very familiar parasite. Infected flies may be found in late spring or early autumn adhering to window-panes or mirrors, surrounded by a dusty halo of discharged spores. In some cases it is possible to find live insects sticking to the glass, trying to break away from the mycelium which surrounds them.

The infection of the fly begins with the adherence of viable spores to the surface of the abdomen where they may go through a period of dormancy or germinate immediately (**6**). The degree of infection depends on the amount of spores picked up by the fly. Several factors may be instrumental in causing conidial formation after a long period of dormancy within the insect host; but experiments performed at Moscow, Idaho, indicate that environmental conditions, mainly humidity, greatly influence conidial formation. These experiments were conducted over a period of seventeen months, during the latter part of 1936 and throughout 1937, experiencing varying weather conditions.

In these experiments several hundred flies were used, and two different methods were employed to minimize contamination by other fungus forms and to induce germination of most of the *Empusa* conidia.

To obtain cultures of the fungus, a modification of the method used by Sawyer (**5**) was employed. Several flies were glued thoroughly to a pane of glass which was then inverted over half a moisture chamber containing moistened cotton. The glass was sealed tightly to the chamber, and the whole apparatus set in a warm, well-lighted spot. The speed with which the spores germinated and mycelium broke from the abdomen of the fly, varied from three to eight days. This may have been due to the varying amounts of spores present within the insect.

The second method employed was the use of several well-known nutrient solutions, distilled water and tap water. As a complete set-up, 100 small vials were half-filled with a nutrient solution and a fly placed in each. In every case similar results were obtained, although much contamination by *Mucor, Rhizopus,* and *Penicillium* resulted. Knop's, Detmer's, Pfeffer's, Crone's (**4**), and Sach's and Czapek's (**3**) nutrient solutions were used. A sugar-starch solution as follows was also employed.

Water	1000 gms.
Soluble starch	2 gms.
Sucrose	1 gm.

Table I shows the relation of *Empusa* infections to the amount of precipitation covering the period during which tests were made. The results can be understood more clearly if the total amount of rainfall and departure from the normal for each month is presented.

TABLE I

RELATION OF *Empusa* INFECTIONS TO TOTAL AMOUNT OF RAINFALL[1] FROM SEPTEMBER 1936 TO NOVEMBER 1937

Month	Total ppt., inches	Departure from normal	Number of flies tested	Number of flies infected	Percentage infection
Sept.	1.18	+0.02	20	1	5.0%
Oct.	0.30	−1.30	40	0	0.0%
Nov.	0.24	+2.78	40	1	2.5%
Dec.	2.74	+0.06	0	—	—
Jan.	3.60	+0.66	0	—	—
Feb.	2.77	+0.62	0	—	—
Mar.	2.25	+0.05	0	—	—
Apr.	3.81	+2.29	0	—	—
May	0.69	−1.19	120	48	40.0%
June	2.92	+1.56	100	48	48.0%
July	0.23	−0.38	0	—	—
Aug.	0.49	−0.23	0	—	—
Sept.	0.79	−0.37	200	3	1.5%
Oct.	1.51	−0.09	200	0	0.0%
Nov.	3.60	+0.58	200	0	0.0%

[1] Precipitation data from Carter (**1, 2**).

In the fall of 1936, Idaho experienced the driest weather in the history of the weather bureau. The results obtained showed that a total of 2 per cent of the flies studied were infected with *Empusa Muscae.* In the winter and spring of 1937, there was a complete reversal of moisture conditions. From January to April, northern

Idaho experienced some of the wettest weather since 1894. During May, although precipitation fell off considerably, the saturated condition of the soil, swollen creeks, and remaining snow, kept the humidity high. Again in June rainfall was very heavy. The wet conditions produced heavy casualties among the house-flies. A total of 220 flies were studied showing the percentage of infection to be 43.6. From July to November the amount of rainfall was very low. The atmosphere as a result was very dry, and although a total of 600 flies were studied only three infections were obtained. The percentage for this period was 0.5.

Direct correlation was found to exist between the amount of precipitation in this area and the *Empusa* infections. During the dry months, few or no infected flies were found and in humid or rainy months an abundance was obtained. The prevalence of *Empusa Muscae* infections, therefore, seems to vary directly with the amount of rainfall.

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A COMPARISON OF MYCETOZOA FAUNA IN SANDSTONE AND LIMESTONE REGIONS OF AUGUSTA COUNTY, VIRGINIA

LLOYD G. CARR

Collecting the Mycetozoa in southeastern Augusta County has disclosed a number of unusual forms, and has revealed that the types of genera present in one region compared to another vary according to the lime content of the soil. This investigation is the first ecological study of the Mycetozoa in relation to their lime requirement.

Forms have been secured from two general localities: one situated in a sandstone region near the base of the Blue Ridge Mountains, the other in a limestone region well out in the Shenandoah Valley. The forms in the sandstone region select habitats on old logs and sphagnum moss which is scattered around the numerous ponds that appear there. Numerous springs feed into these ponds which are formed as a result of limestone sinks. However, there is no outcropping of limestone in the present area as it is completely covered by overthrusting and many feet of talus and wash at the foot of the Blue Ridge. Therefore, the limy material that lies many feet below the sandstone wash is unavailable to the use of the lime genera of the Mycetozoa. The ponds usually present a gradual incline on the sides, and are well hidden in the flat woods. The numerous springs near these ponds form boggy shallows on the edges, affording excellent pockets in which leaves and other vegetable materials collect. In the process of decay these materials hold much moisture. All of these factors are favorable to the growth of the Mycetozoa.

Many of the more familiar species of the Mycetozoa are present here. *Didymium nigripes* var. *xanthopus* Lister grows in abundance on sphagnum moss. *Lamproderma arcyrionema* Rost., *Comatricha typhoides* Rost., *Arcyria denudata* Wettstein, *A. cinerea* Pers., *A. digitata* Rost., *A. nutans* Grev., *Stemonitis*

axifera Macbr., *S. splendens* Rost., *Trichia persimilis* Karst., *T. scabra* Rost., and *Hemitrichia vesparium* Macbr. are familiar forms on decayed logs. A few forms appear to confine themselves to brush and leaf heaps. *Oligonema nitens* Rost. and *Leocarpus fragilis* Rost. select heaps of this type. *Trichia lutescens* Lister appears in the sandstone region representing the first record for its appearance in the east and the second record in North America.[1] It was found on the inner bark of a fallen oak tree which had decayed.

Upon examination of the lime species occurring in the sandstone region, the fructifications are observed to develop in scanty quantities of small sporangia. A small number of lime species was collected over a wide area in the sandstone region. *Physarum columbinum* Sturgis, *P. oblatum* Macbr., *P. viride* Pers., *P. globuliferum* Pers., *Badhamia lilacina* Rost., *B. orbiculata* Rex., *Fuligo septica* Gmelin., *Didymium nigripes* var. *xanthopus*, and *Leocarpus fragilis* Rost. are forms occasionally seen in the region. Also, the lime genus *Diderma* is represented here with a few conspicuous forms, *Diderma floriforme* Pers., *D. radiatum* Morg., *D. testaceum* Pers., and *D. effusum* Morg. It appears that the best material represented in the sandstone region is from the genera incorporating no lime in their structures.

The "lime genera" collected in these regions were *Badhamia* Berk., *Physarum* Pers., *Fuligo* Haller, *Craterium* Trentep., *Leocarpus* Link, *Diderma* Pers., *Diachea* Fries in the Physaraceae, and *Didymium* Schrad. and *Mucilago* Adanson in the Didymiaceae.

Considering the finds from the sandstone region on a percentage basis, it is calculated that 41.9 per cent of the 31 forms collected are included in the "lime general," whereas 58.1 per cent require no lime.

The Shenandoah Valley lies essentially in a limestone region. Here and there hills or monadnocks rise upward from the valley plain. Often the sides of the hills are indented with sinks. Gibson's Hill, near Barterbrook, possesses two sinks. One sink is in the form of a big bowl wooded on each side with oaks, maples, cherries, and the beaked alder. The edges of the bowl slant to a small pool of water. On one edge there are precipitous cliffs with

[1] The only other report for this form in North America is from Colorado.

shelf-like projections. These furnish pockets for the collection of leaves and other vegetable materials which hold considerable moisture. As the water moves over the rocks and seaps through the leaves, it carries with it calcium carbonate which collects in the leaf pockets. Here the lime species of the Mycetozoa are numerous in well-developed, robust fructifications. Several of the rarely collected forms appear in the sink. It is possible that the available quantity of lime and the copious moisture had something to do with their occurrence here.

Physarum Listeri Macbr. is present and has a double sporangial wall consisting entirely of lime.[2] Since the double wall has not been noted previously, the material proves to be the most representative of the form. *Physarum serpula* Morgan, a rare form, *P. contextum* Pers., *P. lateritium* Morg., *P. melleum* Mass., and *P. sinuosum* Link occur here on leaves.

Diderma Trevelyani Fries, a rare form in the east, was found on decayed leaves of *Quercus marilandica* Muench in one of the numerous pockets.[2] When its sporangial wall breaks in a petaloid fashion, it resembles a miniature water lily that has just opened. This species has a three-walled sporangium, and the middle one is studded with crystalline lime so as to form an unbroken wall. As its requirement for lime is rather high, the available lime in a region would determine its distribution. It should be looked for in limestone sinks. *Diderma testaceum* spreads in much larger plasmodiocarps on leaves in the sink than it does in sandstone regions.

Diachea splendens Racib. was fruiting extensively near the base of the sink. This form is not often collected, but may be looked for with some degree of success in limestone sinks. It was found in all of the sinks which I examined. *D. bulbillosa* Lister and *D. leucopoda* Rost. are also present.

Craterium minutum Fries, an odd form, with goblet-shaped fruiting bodies was discovered. A thin coat of powdery lime was noted on the surface of the lids.

Only a few forms requiring no lime have been observed here. These are *Hemitrichia serpula* Rost. and *Arcyria nutans* Grev.

[2] Hagelstein, Robert. Notes on the Mycetozoa. Mycologia **30**: 336–353. 1938.

Determining the percentages, it is found that 11.7 per cent of the 17 forms collected in the limestone region contain no lime in their structures, whereas 88.3 per cent are typical members of the lime genera.

Considering the total number of lime forms, 53.5 per cent were from a small area in the limestone region, whereas 46.5 per cent occurred over an extensive area in the sandstone region. Of the forms requiring no lime, 90 per cent were found in the sandstone areas, while 10 per cent were present in the limestone region.

In connection with this study, Mr. Robert Hagelstein of the New York Botanical Gardens writes: "I have always found that the calcareous forms are in greater abundance and far more robust in limestone regions than in regions without limestone, taking as an example Long Island where I reside, and which is of glacial origin without much lime."

SUMMARY

The results present several interesting facts: the lime loving genera are abundant in limestone regions, while in others they are lacking or are not prolific over wide areas; and, the forms using no lime in their structures appear in abundance in localities where the lime content of the soil is low. It is indicated that the fruiting bodies of the lime genera growing in an abundance of lime are better developed. Limestone sinks are favorable spots where odd and rare forms of the lime genera are localized.

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PORIA ANDERSONII AND POLYPORUS GLOMERATUS, TWO DISTINCT HEART-ROTTING FUNGI

W. A. Campbell & Ross W. Davidson

(with 2 figures)

INTRODUCTION

Pure-culture studies of wood-rotting fungi, which are conducted as an aid in distinguishing macroscopically similar rots and determining their relative importance, frequently also clarify the taxonomic relationships of the species involved. Such is the case with *Poria Andersonii* (Ellis & Ev.) Neuman and *Polyporus glomeratus* Peck, two species frequently isolated in a decay study, which to date have not been clearly separated by mycologists and pathologists.

Peck (**10**) in 1873 described *P. glomeratus* and it has since been recognized by most mycologists as a well-defined species of *Polyporus*. Unfortunately the taxonomic position of *P. Andersonii* has not been so well established. In 1890 Ellis and Everhart (**5**) described *Mucronoporus Andersoni* as a resupinate polypore fruiting under the bark of an "oak log." Only a fragment of the type specimen is still in existence in The New York Botanical Garden and it appears to be as originally described. This fungus has since been confused with *P. glomeratus*. In fact Ellis and Everhart themselves were in part responsible for this confusion because they issued exsiccati sets in which they included a resupinate form of *P. glomeratus*, from maple, under the name *M. Andersoni*. In 1894 Underwood (**11**) described a fungus similar to *M. Andersoni* on the under side of poplar logs in Indiana as *Polyporus* (*Poria*) *xanthosporus*. Murrill (**7**) in 1916 described a new genus *Xanthoporia* based on Ellis and Everhart's *Mucronoporus Andersoni*. Neuman (**8**) listed the Ellis and Everhart species as *Poria Andersonii* and gave *Poria xanththospora* as a synonym. Lloyd at first separated *P. glomeratus* from *P. xanthospora*, as he classified his

specimens, but later (**6**) declared the latter to be a resupinate form of *P. glomeratus*. Baxter (**1**, **2**) apparently came to the same conclusion.

Ellis and Everhart's failure to distinguish between the two species is clearly shown by examination of their exsiccati under the name *Mucronoporus Andersoni*. Their specimen *3101*, North American Fungi, is really *P. glomeratus*, and was collected on *Acer rubrum* L. at Shelpot Creek near Wilmington, Delaware, in 1893. Their number *3101b* in the same series is *P. Andersonii* collected on poplar logs in Ohio, 1895.

CHARACTERISTICS IN NATURE

All specimens of *P. glomeratus* examined by the writers occur on the outside of the bark on maple and beech, usually with some indication of a pileus. On the other hand, *P. Andersonii* apparently always develops under the bark or thin wood layer of dead standing snags or down logs of several hardwood species. Although it is reported on willow, poplar and hickory, it is found most commonly on species of oaks. None of the specimens shows any indication of a pileus. *P. glomeratus* sporophores contain easily demonstrated large setal hyphae in the trama of the tubes (FIG. 1, *G*), which are illustrated by Overholts (**9**), whereas *P. Andersonii* has much smaller setal hyphae (FIG. 1, *C*). In addition, the distribution of such setal hyphae in sporophores of *P. Andersonii* is more erratic and in most cases difficult to demonstrate.

Several specimens of *Poria Leei* (Murr.) Sacc. & Trott., collected on oaks in California, were examined and the sporophores appeared to be identical with *P. Andersonii*. Isolations made from a sporophore collected on tanbark oak by E. E. Morse near Berkeley, California, were similar in all respects to those secured from *P. Andersonii* in the East.[1]

P. Andersonii resembles, in manner of fruiting and in appearance of the sporophores, the *Poria* recently described by Campbell and Davidson (**4**) as the cause of the sterile conks on birch.[2]

[1] This specimen was sent originally to L. O. Overholts who forwarded a portion to the writers for culturing.

[2] There seems to be little question but that this *Poria* is the same as *Poria obliqua* (Pers.) Bres. found on birch and other hosts in Europe.

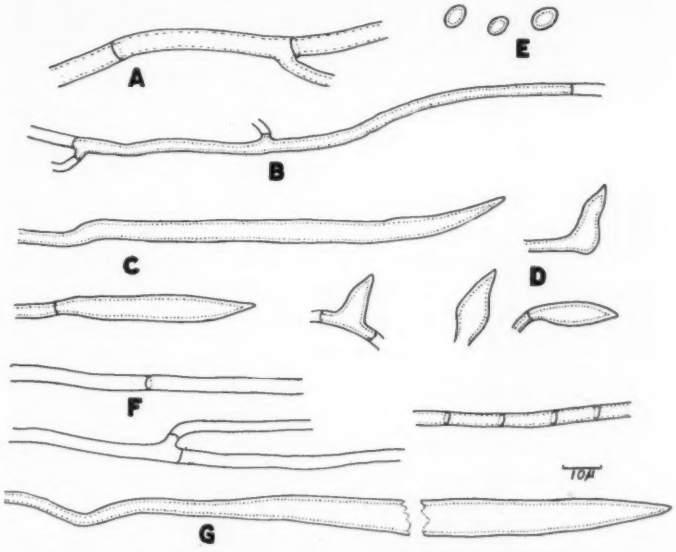

FIG. 1. *A–E, Poria Andersonii. A,* submerged hyphae; *B,* superficial hyphae; *C,* setal hyphae; *D,* bulbous setae; *E,* basidiospores. *F* and *G, Polyporus glomeratus. F,* submerged hyphae; *G,* setal hyphae.

However, *P. Andersonii* has yellowish-green spores, 5–8 × 4–5 μ and spores of the latter are hyaline or only occasionally pale-yellow, 6–10 × 4–6 μ. The two species are also very different in culture.

CULTURAL CHARACTERISTICS AND DISTRIBUTION

PORIA ANDERSONII

Petri-dish cultures: Growth slow forming in 7 days a mat 2–3 cm. in diameter.[3] Mat either cottony or silky-cottony, thin, and not raised above the agar or fine woolly to felty, raised and compacted, primuline yellow,[4] mustard yellow and apricot yellow to as dark as antimony-yellow and yellow-ochre.

[3] All descriptions based on mats grown on 1.5 per cent Difco malt with 2 per cent agar, in diffused light at room temperature averaging approximately 26° C.

[4] Ridgway, R. Color standards and color nomenclature. 1914.

In 14 days mat 4.5–7 cm. in diameter (FIG. 2, *A, B*), very variable, usually raised, compacted, with definite concentric rings, often showing pronounced segments, mostly felty but occasionally long cottony about inoculum; light-orange-yellow to yellow-ochre, raw sienna and buckthorn-brown; margin either rising abruptly from the agar or with a thin, appressed, yellowish or almost colorless zone of advancing growth. Marginal characters very variable as some cultures may show thin marginal growth and dark colored staled areas over all or only a portion of the surface.

Hyphae staining with eosin, 2–5 μ in diameter (FIG. 1, *A*), no clamps; from staled areas under center, yellowish, collapsed, non-staining, with numerous cross-walls and often broken up into irregular lengths; fibrous hyphae thick-walled, dark brown, 2–4(–5) μ in diameter (FIG 1, *B*); long dark-brown setal hyphae common in some cultures, rare in others, up to 8 μ in diameter and 250 μ long (FIG. 1, *C*); bulbous setae common in compacted, staled areas and in fruiting structures (FIG. 1, *D*); basidiospores occasionally formed in poroid areas, ellipsoid, smooth, yellowish-green, 5–8 $\times$ 4–5 μ (FIG. 1, *E*).

Temperature relations: Optimum temperature for growth approximately 35° C. Average diameter of mats in 7 days at constant temperatures,[5] in dark, as follows: 1.0 cm., 20°; 3.1 cm., 25°; 5.2 cm., 30°; 5.5 cm., 35°; slight growth, 40°.

Test-tube cultures: In 14 days mat raised, fine woolly to felty, or appressed-felty, mustard-yellow and apricot-yellow to as dark as antimony-yellow and yellow-ochre.

In 28 days mat on slant compacted woolly to felty, yellow-ochre to buckthorn-brown and raw sienna; on agar cylinder lighter, mustard-yellow to antimony-yellow and yellow-ochre, often with pronounced zones, tough, forming a heavy film which is difficult to tear with a needle. An occasional isolate will form well-developed pores on a raised mycelial cushion at the lower end of the slant.

Flask cultures: The mycelium, on maple and oak blocks, is at first yellow, cottony, becoming in 10 months compacted ochraceous

[5] Dishes kept 1 day at room temperature before placing in incubator. To prevent drying of the medium, especially at high temperatures, the Petri dishes were placed, 4 to a can, in tins, with tight-fitting lids.

buff, cinnamon or yellow-ochre to as dark as buckthorn-brown or ochraceous tawny; irregular areas outlined by narrow black lines rather common. Sporophores are formed in some flasks, usually where the mycelium makes contact with the glass. The sporophores are much distorted by pressure against the glass and produce basidiospores in sufficient abundance as to cause a yellowish-green spore print. Wood decayed by *P. Andersonii* becomes very soft, white and spongy.

DISTRIBUTION

Ariz., *Quercus Gambelli* Nutt.; Ark., *Quercus* sp.; Calif., *Q. lobata* Née; Ind., *Q. rubra* L.; Kans., host not known; Md., *Quercus* sp. and *Salix nigra* Marshall; Mo., *Q. marilandica* Muench.; N. C., *Quercus* sp.; Ohio, *Populus* sp.; Ore., *Q. garryana* Doug.; Pa., *Carya* sp. and *Quercus* sp.; Texas, host not known; Va., *Quercus* sp.; and Wis., *Quercus* sp.

Cultures have been obtained from *Quercus* sp. from the following states: Calif., Md., Mo., Ohio and Va. From *Carya* sp.: Iowa and Ohio.

POLYPORUS GLOMERATUS

Petri-dish cultures: Growth very slow-forming in 7 days a mat 1–1.5 cm. or less in diameter. Mat usually closely appressed with a thin, whitish to yellowish covering over the inoculum, and a colorless zone of advancing growth; often with a distinct brown staled area under the mat. An occasional culture forms a white, cottony or woolly mound over the inoculum with no trace of staling.

In 14 days mat 2–4 cm. in diameter, usually appressed, thin, indeterminate in color about the margin and with a brown staled area under the center (FIG. 2, *D*); surface hyphae somewhat velvety or compacted over the inoculum; white or colorless at margin to primuline-yellow, olive-ochre or dark-olive-buff at center. An occasional culture fails to develop the brown staled area and becomes raised, thick, felty, white at margin to olive-buff and deep-olive-buff at center (FIG. 2, *C*).

Submerged hyphae either hyaline, staining deeply with eosin or yellowish, non-staining, 2–5 μ in diameter, without clamps; in

staled areas thick-walled, broken-up into irregular shapes often with short, connected, swollen cells (FIG. 1, *F*); fibrous hyphae brown, thick-walled, 1–.5 μ in diameter; dark-brown, opaque, setal

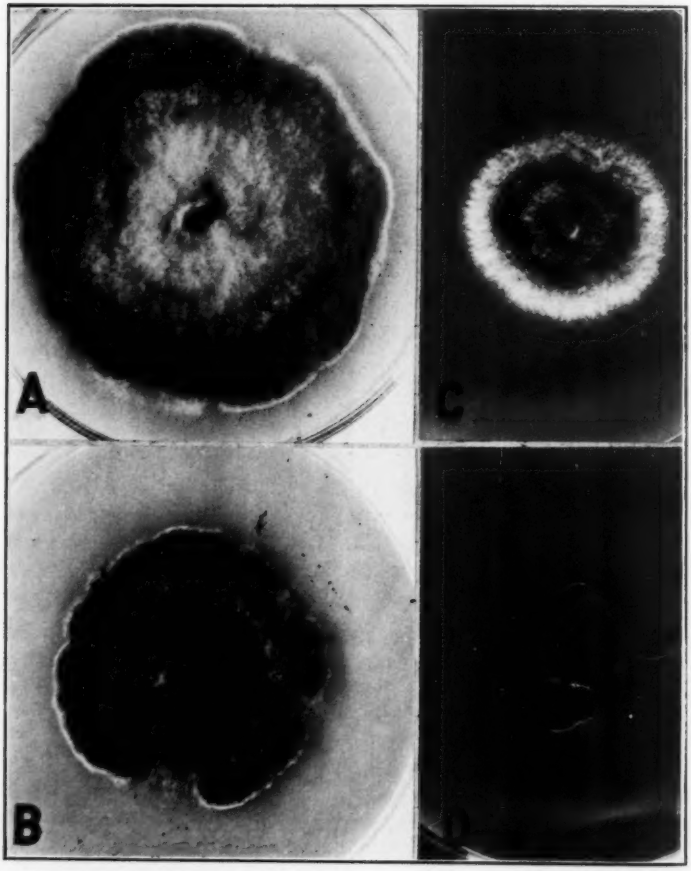

FIG. 2. *A* and *B*, petri-dish cultures age 14 days of *Poria Andersonii*. *C* and *D*, petri-dish cultures age 14 days of *Polyporus glomeratus*.

hyphae usually present in cultures 14 days old but sometimes lacking, 7–16 μ in diameter and 150–400 μ long (FIG. 1, *G*).

Temperature relations: Optimum temperature for growth approximately 25° C.

Test tube cultures: In 14 days appearance of different cultures rather variable, either slow-growing, appressed, with dark-brown staled areas under the mat and with scant white to olive-ochre thin cottony, or velvety, superficial growth; or raised, thick woolly on slant to cottony on agar cylinder and lacking the staled area under the mat; white at margin to buffy brown on slant with a trace of olive-ochre; setae appearing as radiating streaks against the glass, visible with the naked eye and very prominent under a lens.

In 28 days mat darker, from old-gold to yellow-ochre and buckthorn-brown usually with dark-brown staled area extending beyond the margins of growth.

Flask cultures: On hard maple blocks in wide mouth flasks, *P. glomeratus* forms a luxuriant cottony mycelium, much heavier and more robust than on malt agar. Small abortive sporophores form in from 7 to 10 months. The fungus makes little growth on oak heartwood but develops a moderately heavy mycelium on the sapwood. Sporophore formation takes place in 6–8 months with fairly large, well-formed fruiting bodies, producing yellowish-green spores in abundance, formed on the top or sides of the oak blocks. The oak sapwood is well decayed but the heartwood is only slightly attacked.

DISTRIBUTION

Cultures obtained from decay studies indicate that the fungus causes a trunk rot in living *Acer Saccharum* Marshall, and *A. rubrum* L. in Conn., Mass., Mich., Penn., W. Va., Va., and Wis. In addition, cultures have been obtained from a sporophore on beech collected in Mass.

CULTURAL DIFFERENCES

The mats formed by the two fungi, on malt agar, are entirely dissimilar as to general appearance. *P. Andersonii* is typically yellow at all ages, and is usually woolly or felty in texture. *P. glomeratus* commonly starts as a white mycelium about the inoculum becoming compacted, olive-buff. In addition the former grows 2 to 3 times as fast and has an optimum temperature of approximately 35° C. in contrast to an optimum of approximately 25° for *P. glomeratus*. In both species setal hyphae are formed

in culture, but those produced by *P. glomeratus* are usually larger, more opaque, typically from 7–16 μ in diameter, while those of *P. Andersonii* usually range from 5–8 μ. In addition, the latter produces short bulbous setae on the mat surface, especially in staled areas. This form is rarely found in *P. glomeratus* cultures.

ECONOMIC IMPORTANCE

Poria Andersonii is one of the fungi commonly isolated from trunk decay of living oak trees throughout the middle western states. It usually enters through branch stubs but it may also enter through fire-scars or other injuries. It causes a white decay similar to that caused by *Fomes Everhartii*.

Polyporus glomeratus causes a white to light-brown spongy heart rot of maples and is common (especially on sugar maple) in some areas in the Lake States.[6]

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NEW SPECIES OF UREDINALES[1]

GEORGE B. CUMMINS

(WITH 6 FIGURES)

Puccinia incallida Cumm. sp. nov. (FIG. 2)

Pycniis epiphyllis, globosis, 90–120 μ diam., paucis. Aeciis epiphyllis, uredinoidis, minutis, flavidis, aggregatis in maculis flavis 0.5–2.0 mm. diam.; aeciosporis ellipsoideis vel obovoideis, 23–30 × 29–40 μ; membrana 2–2.5 μ cr., cinnamomeo- vel aureo-brunnea, valide aculeata (3 μ), poris germ. obscuris (3 aequatorialibus?). Urediis ignotis. Teliis ignotis; teliosporis in aeciis ellipsoideis, utrinque rotundatis, ad septum constrictis, 19–23 × 40–55 μ; membrana aequaliter 1–1.5 μ cr., hyalina, levi; pedicellis hyalinis, brevibus.

On *Gouania longipetala*, between Suhum and Apedwa, Gold Coast, Africa, July 28, 1937, *F. C. Deighton CB846*. Type in the Arthur Herbarium, Purdue University Agricultural Experiment Station and the Herbarium of the Imperial Mycological Institute.

Puccinia incallida appears to be closely related to *P. paraensis* Diet. from Brazil but differs in lacking paraphyses and in having larger teliospores.

Puccinia subtegulanea Cumm. sp. nov. (FIG. 1)

Pycniis et aeciis ignotis. Urediis hypophyllis, cinnamomeo-brunneis, aggregatis vel confluentibus, ellipticis vel linearibus, 0.5–2 mm. longis epidermide bullata dein longitudinaliter fissa diu tectis; urediosporis ellipsoideis vel obovoideis, 11–15 × 15–19 μ; membrana 1 μ cr., cinnamomeo-brunnea, dense et minute echinulata, poris germ. 2, aequatorialibus. Teliis hypophyllis, subepidermalibus, dense aggregatis, atris, multiloculatis, indehiscentibus, soris individuis minutis, paraphysibus numerosis coalitis; teliosporae variabiles, oblongae vel clavatae, ad apicem obtusae, rotundatae vel rostratae, ad basim attenuatae, medio non vel leniter constrictae, 10–16 × 28–40 μ; membrana 1 μ cr., ad apicem 3–6 μ, castaneo-brunnea, levi; pedicellis brevibus, fulvis.

On *Mariscus umbellatus*, Akwadum, Gold Coast, Africa, August 17, 1937, *F. C. Deighton CB910*. Type deposited in the Arthur

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Herbarium, Purdue University Agricultural Experiment Station and the Herbarium of the Imperial Mycological Institute.

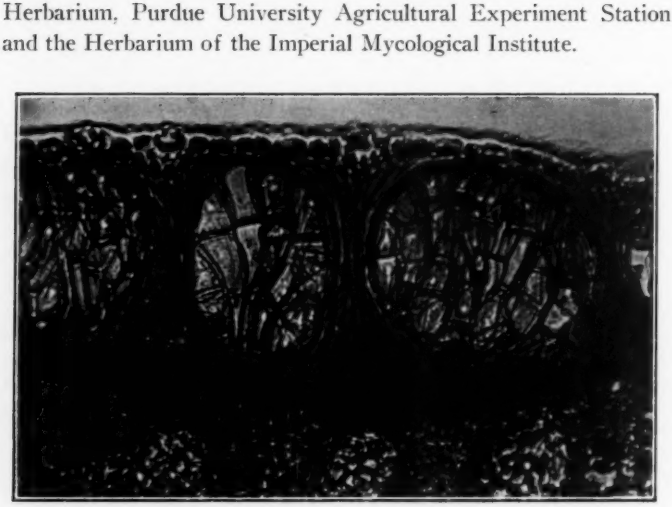


FIG. 1. *Puccinia subtegulanea*, showing loculate, indehiscent telia. × 500.

This species is similar in general to the group of species which has two germpores in the urediospores and indehiscent loculate telia, but has smaller spores than any previously described.

Puccinia Thelypodii Cumm. sp. nov. (FIG. 3)

Pycniis globosis, 90–130 μ diam., sparsis. Aeciis plerumque per totam foliorum superficiem inferiorem aeque distributis vel rarius aggregatis, margine recurvato; cellulis peridii 16–19 × 22–29 μ, pariete interiore verrucoso 2–3 μ cr., exteriore striato 5–7 μ cr.; aeciosporis ellipsoideis vel globosis 13–16 × 16–19 μ; membrana hyalina 1 μ cr., minuteque verrucosa. Uredііs sparsis, rotundatis, 0.3–1.0 mm. diam., hypophyllis vel rarius epiphyllis, pulverulentis, cinnamomeo-brunneis; urediosporis late ellipsoideis vel globosis, 18–20 × 20–26 μ; membrana 1.5–2 μ cr., cinnamomeo-brunnea, dense et minuteque echinulata, poris germ. 2 aequatorialibus. Teliis caulicolis et fructicolis, ellipsoideis vel rotundatis, 0.3–2.0 mm. longis, pulvinatis, atro-brunneis; teliosporae clavatae, oblongae vel ellipsoideae, ad apicem rotundatae, truncatae vel attenuatae, ad basim attenuatae, medio constrictae, 19–24 × 39–58 μ; membrana castaneo-brunnea, 1.5–2.5 μ cr., ad apicem 5–10 μ cr., levi; pedicello hyalino vel apice flavidulo, persistenti, 6–10 × 50–90 μ.

On *Thelypodium lasiophyllum*, Soboba Hot Springs, Riverside County, California, February 1938, *H. E. Parks 6154*. Type in

the Arthur Herbarium, Purdue University Agricultural Experiment Station.

When Mr. Parks sent this specimen he wrote that he believed telia on the old plants were inoculating the seedlings which bore the aecia and uredia. His suggestion was verified by cultures using plants raised from seed in the greenhouse at Lafayette, Indiana, and subjected to inoculation by germinating teliospores on the old stems sent with the original collection. Inoculations made April 21 and May 6 produced pycnia May 1 and May 16 and aecia May 5 and May 22, respectively. The species is thus autoecious and macrocyclic.

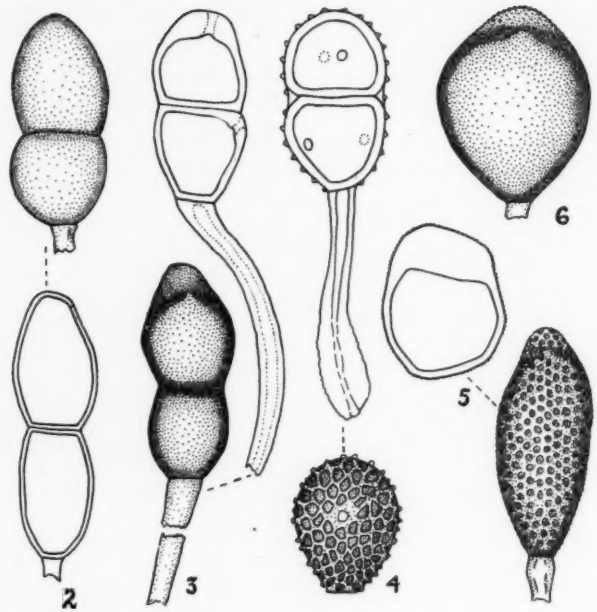
Uropyxis reticulata Cumm. sp. nov. (FIG. 4)

Pycniis et aeciis ignotis. Urediis subepidermicis hypophyllis, sparsis, minutis, 0.1–0.3 mm. diam. vel ramicolis in incrassationibus elongatis fusiformibus copiose ortis, brunneis; urediosporae obovoideae, 16–22 × 23–27 μ; membrana 2 μ cr., cinnamomeo-brunnea, reticulata, poris germ. 2, aequatorialibus. Teliis conformibus; teliosporae ellipsoideae vel oblongae, utrinque rotundatae, medio leniter constrictae, 18–23 × 31–40 μ; membrana 2.5–3 μ cr., cinnamomeo- vel castaneo-brunnea, verrucosa, quaque cellula poris binis mediis praedita; pedicello persistenti, sporam aequante, deorsum lenissime incrassato.

On *Bignonia Unguis-cati*, La Plata, Argentina, February 1937, *Juan C. Lindquist* (type); La Plata, Argentina, May 1920, *C. Spegazzini*. Type in the Arthur Herbarium, Purdue University Agricultural Experiment Station and the Museo de La Plata, La Plata, Argentina.

Spegazzini (Rev. Argent. Bot. **1**: 108. 1925) first reported this rust as *Puccinia Bignoniacearum* Speg., an entirely different species which belongs in the genus *Prospodium*. His specimen, kindly loaned to me by Dr. Lindquist, consists of foliicolous sori, while Lindquist's later collection consists of the ramicolous form and is here taken as the type.

The species is unique in developing urediospores with reticulately sculptured walls and has only two equatorial pores, while *Uropyxis* is characterized by urediospores having several scattered pores. Although lamination of the walls of the teliospores is observable only at the septum the presence of two pores in each cell clearly indicates the genus *Uropyxis*.



FIGS. 2–6. 2, teliospores of *Puccinia incallida;* 3, teliospores of *Puccinia Thelypodii;* 4, one teliospore and one urediospore of *Uropyxis reticulata;* 5, one aeciospore and one teliospore of *Uromyces evastigatus;* 6, one teliospore of *Uromyces dilucidus.* (All figures from type specimens. × 650.)

Uropyxis Rickiana Magn., also on some bignoniaceous host, likewise causes hypertrophy and has similar teliospores. I have not seen this species but since no urediospores were described it seems advisable to keep *U. reticulata* separate.

Uromyces dilucidus Cumm. sp. nov. (FIG. 6)

Pycniis, aeciis et urediis ignotis. Teliis amphigenis, 0.2–0.5 × 0.5–1.5 mm., sparsis vel aggregatis, pulverulentis, castaneo-brunneis; teliosporis globoso-ellipsoideis vel ovatis, apice rotundatis, papilla subhyalina verruculosa usque 5 μ alta instructis, 29–33 × 32–44 μ; membrana cinnamomeo- vel castaneo-brunnea, 2–3 μ cr., levi; pedicello hyalino, brevi, deciduo.

On *Sisyrinchium striatum,* Tunuyán, Mendoza, Argentina, March 8, 1933, *Ruiz Leal 1273.* Type in the Arthur Herbarium,

Purdue University Agricultural Experiment Station and the Museo de La Plata, La Plata, Argentina.

Although only telia are present in this collection it is not probable that the species is microcyclic. *Uromyces dilucidus* is distinctive because of the large teliospores.

Uromyces evastigatus Cumm. sp. nov. (FIG. 5)

(*Pucciniola Urbaniana* Arth. N. Am. Flora **7**: 795. 1926. Not *Uromyces Urbanianus* P. Henn. 1897.)

Pycniis paucis amphigenis, profunde immersis, 120–150 × 180–250 μ, maculis incrassatulis usque 1.5 cm. diam. occupantibus. Aeciis cupulatis, gregariis, inter pycnia sparsis, plus minusve profunde immersis, 0.3–0.4 mm. diam., margine lacerato; cellulis peridii 18–27 × 35–55 μ, pariete interiore rugoso 2–2.5 μ cr., exteriore striato 3–6 μ cr.; aeciosporis oblongis, ellipsoideis vel angulato-globosis 19–27 × 29–41 μ; membrana 1.5–2.5 μ cr., ad apicem 5–12 μ, hyalina vel flavida, minuteque verruculosa. Urediis nullis. Teliis amphigenis, aggregatis, 1–2 mm. diam., pulverulentis, castaneo-brunneis; teliosporis oblongis vel ellipsoideis, apice rotundatis vel attenuatis, 20–25 × 39–53 μ; membrana castaneo-brunnea 2–3 μ cr., ad apicem 6–9 μ, reticulata (1–1.5 μ diam.); pedicello brevi, hyalino.

On *Phthirusa pyrifolia*, vicinity of San Salvador, El Salvador, March 30–April 24, 1922, *Paul C. Standley 23106* (type); vicinity of Tonacatepeque, Dept. San Salvador, El Salvador, December 30, 31, 1921, *Paul C. Standley 19473*. Type in the Arthur Herbarium, Purdue University Agricultural Experiment Station.

Uromyces evastigatus differs from *U. Urbanianus* P. Henn., to which Arthur (*l.c.*) originally assigned the above collection but under the now abandoned genus *Pucciniola*, in having larger and more coarsely sculptured teliospores.

During this study it was found that in several species described as having verrucose teliospores the sculpture is actually reticulate, although very finely so. The following key, based upon this study, gives differential characters for the species of *Uromyces* on Loranthaceae:

Teliospores longitudinally ridged.
  Teliospore-pedicel long, rugosely inflated. .....*U. ornatipes* Arth.
  Teliospore-pedicel short, fragile.
    Teliospores large, 38–50 μ long. .....*U. euphlebius* Sydow.
    Teliospores smaller, 32–37 μ long. .....*U. socius* A. & H.

Teliospores reticulate, sometimes striately so.
Species macrocyclic.
Urediospores echinulate. .....*U. Loranthi* J. & H.
Urediospores longitudinally ridged. .....*U. Phtirusae* Mayor.
Species demicyclic.
Reticulation obscure; pits 0.5–0.8 μ diam.
Teliospores ellipsoid, 18–23 × 29–35 μ..*U. circumscriptus* Neger.
Teliospores oblong-ellipsoid, 15–23 × 33–43 μ.
U. Urbanianus P. Henn.
Reticulation obvious; pits 1–1.5 μ diam.
Teliospores oblong-ellipsoid, 20–25 × 39–53 μ.
U. evastigatus Cumm.

The specimens of *Puccinia incallida* and *P. subtegulanea* were received from Dr. G. R. Bisby, the Imperial Mycological Institute, Kew, England, of *Uropyxis reticulata* and *Uromyces dilucidus* from Dr. Juan C. Lindquist, Museo de La Plata, La Plata, Argentina, and of *Puccinia Thelypodii* from Mr. H. E. Parks, Trinidad, California.

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THE GENERA, SKIERKA AND CTENODERMA

E. B. MAINS [1]

(WITH 14 FIGURES)

The genus *Skierka* was described by Raciborski (**11**) in 1900 and was based on the species *Skierka Canarii*. The teliospores were described as one-celled, fusoid, having acuminate apices, without pedicels, and issuing from the telium in a *Cronartium*-like column. Four other species have been described, *S. congonensis* from Africa by P. Hennings (**9**) in 1907, *S. Agallochoa* from Java by Raciborski (**12**) in 1909, *S. Holwayi* from Central America by Arthur (**1**) in 1918 and *S. robusta* from Africa by Doidge in 1926.

During the summer of 1936, the writer collected a rust on *Cupania belizensis* in the El Cayo District of British Honduras.[2] This proved to be the telial stage of a species of *Skierka*. Species of *Skierka* have not been reported on species of *Cupania*. However, *Ctenoderma cristata* has been reported on several species of *Cupania* from Tropical America. A study of collections [3] of this rust has resulted in an interesting discovery. The genus *Ctenoderma* was described by the Sydows (**17**) in 1919, *Ctenoderma cristata* (Speg.) Sydow (*Uredo cristata* Speg.) being cited as the type species. The Sydows concluded that the spores which had been described as urediniospores were teliospores. These were described as one-celled, the wall of two layers, the outer gela-

[1] Papers of the Department of Botany and Herbarium of the University of Michigan.

[2] This expedition was part of a study of the biology of the Maya area, a coöperative study between the University of Michigan and the Carnegie Institution of Washington and was supported by funds from the Horace H. Rackham School of Graduate Study.

[3] Through the kindness of Dr. George B. Cummins the specimens of species of *Skierka* and *Ctenoderma* in the Arthur Herbarium have been available for study. The writer is also indebted to the Director of the Royal Botanical Gardens Kew for loans of types of *Ctenoderma Petchii* and *C. Diploglottidis*.

tinous and thickening above. The species *Ctenoderma cristata* has this spore stage associated with pycnia in small hypertrophied areas. Among the collections available for this study two were discovered which bore abundant scattered telia, typical of *Skierka,* associated with this stage. From these specimens it seems evident that the stage described as telial for *Ctenoderma* is in reality the uredinial. A comparative study of species of *Ctenoderma* with species of *Skierka* has resulted in the conclusion that the former should be included in the genus *Skierka.*

Those species of *Skierka* for which telia are known show considerable similarity in the telial stages (FIG. 9–14), all having fusoid, thin-walled spores which adhere and are forced out of the narrow opening of the telium in columns. There is a greater variation in the uredinial stage. However, a certain similarity occurs throughout the species. They all show more or less lateral thickening of the wall (FIG. 1–8), forming two opposite lateral ridges or plates. This thickening surrounds the spore longitudinally except for the hilum. In *Skierka congonensis* (FIG. 1) it is inconspicuous, the wall of the spore gradually increasing in thickness from 1–1.5 μ on the two faces to 4–6 μ in the lateral longitudinal ridges, the spores therefore being only slightly broader and the wall thicker with the ridges in optical plane than when in surface view. The echinulations of the spore are in longitudinal rows, the rows on the edges of the ridges not differing conspicuously from those on the face. In *Skierka Canarii* (FIG. 3) the wall of the urediniospore shows two layers. The outer hyaline wall is thickened to form a wing-like ridge surrounding the spore longitudinally except for the hilum. The echinulations are arranged in longitudinal rows. Those on the edges of the wings are modified to form a fringe of close set, curving teeth. In *Ctenoderma cristata* (FIG. 4), the outer wall of the urediniospores swells to form a thick plate longitudinally surrounding the spore except for the hilum. With the thickening seen in optical section, the spores are much wider than when in surface view. The two edges of each of the lateral wings are crenate or serrate. The urediniospores of *Ctenoderma Diploglottidis* (FIG. 5) resemble those of *C. cristata.* In *Skierka Holwayi* (FIG. 7) the thickening of the wall reaches its maximum development. The hyaline outer

wall swells to form a very broad wing-like plate surrounding the spore longitudinally except for the hilum. The spore is therefore, considerably wider with the plate in the optical plane than when on edge. The edges of the plate are irregularly crenate. *Ctenoderma Petchii* (FIG. 6) has urediniospores which approach those of *S. Holwayi*.

The relationship of the genus *Skierka* to other genera of the rusts is uncertain. From herbarium specimens it is very difficult to determine accurately the manner of development of the teliospores. The teliospores more or less adhere latrally and tend to form long threads or columns. In *Skierka Holwayi* the adherence is very pronounced and long columns are produced which do not break up easily. In the other species it is less pronounced and the threads or columns may disintegrate in water if some pressure is applied. The teliospores are not catenulate. As Raciborski (**11**) has pointed out, the younger develop between the older, adhering to them and pushing them upward. The teliospores are borne on small globoid cells and at maturity usually separate from them. When the spores are removed from the telium with a scalpel an occasional spore is found with torn remnants of the basal cell adhering. It is possible that such spores are immature and their forcible removal may have ruptured the thin-walled basal cells. There is no well differentiated pedicel and the teliospores have usually been described as sessile. The Sydows (**20**) and Dietel (**7**) emphasize the position of the uredinia and telia stating that they develop beneath stomata. Although this may be true it is not very evident. The sori are deep-seated. They remain covered by the epidermis and in some instances by cells beneath the epidermis, usually there is a layer of compacted hyphae lining the overarching epidermis. The sori finally open by a pore or small slit.

Although the teliospores have usually been considered sessile, *Skierka* has been placed in the Pucciniaceae by most authors. Raciborski (**11**) believed that it was most closely related to *Hamaspora*. Other than in the shape of the teliospores there is very little similarity, *Hamaspora* having free, 2–4-celled teliospores with very long pedicels. The Sydows (**20**) have placed *Skierka* in the Pucciniaceae stating that in spite of the sessile, adhering teliospores,

it is a *Uromyces*-like rust with fusoid teliospores. However, they were uncertain concerning its position in this family.

Arthur (**3**) has proposed a subfamily Skierkatae of the Aecidiaceae (Pucciniaceae) in which he included *Skierka, Ctenoderma, Sphenospora* and *Chaconia*. In *Sphenospora*, the teliospores are free from each other, two-celled with vertical septa and remain attached to well-developed pedicels. In *Chaconia* the teliospores are borne in clusters on a well-developed basal cell and are otherwise free from each other. There are a number of clusters in a telium and these also are free from each other. There seems to be very little evidence of a close relationship of these two genera to *Skierka*.

Dietel (**8**) has placed *Skierka* by itself in the tribe Skierkeae of the Pucciniaceae. He (**7**) states that the development of sori beneath stomata, the adherence of spores forming sporehorns, the cresting of the urediniospores, and the colorless condition of the teliospores are characters found in *Uredinopsis* and therefore concludes that *Skierka* must belong to the oldest group of the Pucciniaceae. Because the members of the tribe Hemileieae of the Pucciniaceae develop their sori in relation to the stomata of their hosts, he would place the tribe Skierkeae near the Hemileieae. Also he suggests that *Skierka* may not be far from the genus *Spirechina*. The development of sporehorns in *Skierka* is most pronounced in the telia and is found in the uredinia only in *S. Holwayi* and there to a limited extent. The sporehorns of *Uredinopsis* are limited to the uredinia. The telial stage of *Skierka* is very different from that of *Uredinopsis*. It seems doubtful whether the suggested similarities to *Uredinopsis* necessarily indicate a position near the beginning of the Pucciniaceae. The relationship to the Hemileieae is also not evident. In the species of that tribe as recognized by Dietel, the spores are pedicellate, the pedicels projecting through the stomata bearing spores outside the host. In *Skierka* the spores develop within the host in deep seated sori, and they are forced out through small openings in the epidermis by the development of younger spores. Also the relationship to *Spirechina* is not very close since in that genus the teliospores are borne on well developed pedicels and are free from each other. If *Skierka* belongs in the Pucciniaceae it would seem to be more

closely related to *Chrysocelis* and *Bitzea* which have sessile, colorless, thin-walled teliospores. The teliospores of *Chrysocelis* and *Bitzea,* however, remain attached to the basal cells and are otherwise free from each other.

The possibility that *Skierka* may belong in the Melampsoraceae should not be disregarded. Apparently only Koorders (**10**) has placed it in this family and he does not discuss his reasons for so doing. The absence of pedicels and lateral adherence of the teliospores have been considered the principal differentiating characters of the Melampsoraceae. The external resemblance of the telia of *Skierka* to those of *Cronartium* has been noted but has been considered a superficial similarity. Apparently one of the principal reasons for excluding *Skierka* from this family is the non-catenulate arrangement of the teliospores. However, *Phakopsora* which has been placed by Dietel (**8**) in the Cronartieae next to *Cronartium* has telia in which the spores are not catenulate, the younger developing between the older. This genus forms compact crusts of brown-walled spores not columns of hyaline spores.

It seems evident that *Skierka* represents a distinct line of development and should be placed in a tribe Skierkeae by itself. With the present two family separation in the Uredinales, the information now available concerning this genus would appear to point as much toward a position near the Cronartieae in the Melampsoraceae as toward the Pucciniaceae. It is evident that a realignment of the genera of the Uredinales is necessary and it is probable that then *Skierka* will fall in a family intermediate between the Melampsoraceae and the Pucciniaceae.

SKIERKA Racib. Parasitische Algen und Pilze Javas II p. 30. 1900.
Ctenoderma Sydow, Ann. Myc. **17**: 102. 1920.

Pycnia subepidermal, sometimes deep-seated in hypertrophied areas; uredinia subepidermal, deep-seated opening by a pore, urediniospores with the wall thickened into two opposite longitudinal ridges or bands, the thickenings often swelling considerably in water; telia subepidermal, deep-seated, opening by a pore; teliospores fusoid, the wall colorless, often showing two layers, the outer separating from the inner, the younger teliospores developing between the older, adhering to them and the spore mass usually pushing out of the telium as a thread or column.

TYPE SPECIES, *Skierka Canarii* Racib.

SKIERKA CONGONENSIS P. Henn. Ann. Musee Congo Bot. V. 2: 90. 1900.

Uredinia hypophyllous, subepidermal, in small groups, pulverulent; urediniospores narrowly ellipsoid or ellipsoid-fusoid, 12–16 × 24–36 μ, the wall 1.5 μ thick, at the apex up to 5 μ and in two lateral longitudinal ridges up to 4 μ, a row of coarse-echinulations on the edge of the ridges, elsewhere moderately echinulate in longitudinal lines, the pores obscure (FIG. 1).

Telia hypophyllous, subepidermal, in small groups, covered by the epidermis except for a small slit or pore, the teliospores adhering and forced out in very long, delicate white threads; teliospores fusoid, 7–10 × 60–80 μ, very acuminate at the apex, truncate at the base, the wall thin, 1 μ, smooth, hyaline, separating into two layers (FIG. 9).

SPECIMENS STUDIED:

Alchornea cordifolia Muell. Njala, Sierra Leone, Dec. 13, 1935, F. C. Deighton, *930*.

Macaranga sp. Kisautu, Congo, Feb. 23, 1907, H. Vanderyst.

Skierka philippinensis sp. nov.

Urediniis hypophyllis, subepidermalibus, diu epidermide tectis, sparsis, 0.2–0.5 mm. diam.; urediniosporis ellipsoideis vel ellipsoideo-fusoideis, 12–16 × 28–54 μ, membramis 1–1.5 μ crassis, lateraliter 4–6 μ, formantibus juga, moderate echinulatis, poris indistinctis; teliis hypophyllis, subepidermalibus, diu epidermide tectis, sparsis; teliosporis coalitis formantibus columellas, fusoideis, 11–19 × 64–110 μ, membranis hyalinis, 1.5–2.5 μ crassis. Specimen typicum in foliis *Canarii luzonici*, Bambong, Nueva Viscaya, Luzon, Philippines, Jan. 1924, Clemens, *1720*.

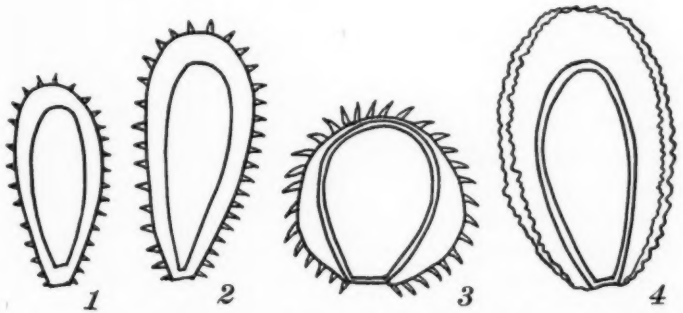
Uredinia hypophyllous, scattered, 0.2–0.5 mm. across, subepidermal, covered by the epidermis and a thin layer of compacted hyphae except for a small pore or slit, pulverulent; urediniospores ellipsoid or ellipsoid-fusoid 12–16 × 28–54 μ, the wall 1–1.5 μ, thickening laterally 4–6 μ in a band extending longitudinally around the spore except for the hilum, moderately echinulate in longitudinal lines, pores obscure (FIG. 2).

Telia hypophyllous, scattered 0.2–0.5 mm. across, subepidermal, similar to the uredinia, the teliospores adhering and forced out in irregular loose columns; teliospores fusoid, 11–19 × 64–110 μ, the wall smooth, hyaline, 1.5–2.5 μ thick, the outer layer often separating from the inner, the apex long acuminate, the base truncate (FIG. 10).

SPECIMENS EXAMINED:

Canarium luzonicum A. Gray, Bambang, Nueva Viscaya, Luzon, Philippine Islands, Jan. 1924, Clemens, *1720,* type (deposited in the Arthur Herbarium, Purdue University and the Herbarium of the University of Michigan.

Canarium sp. Pangasinan, Luzon, Philippine Islands, Feb. 1–14, 1925, Clemens, *5912*; Quemburg Mission, Morobe, New Guinea, Dec. 11, 1935, Clemens, *1327*; March 23, 1936, Clemens, *2160*.



FIGS. 1–4. Urediniospores of species of *Skierka* with lateral thickenings of the wall in the optical plane, × 600; 1, *Skierka congonensis;* 2, *S. philippinensis;* 3, *S. Canarii;* 4, *S. cristata.*

Collections of this species have been identified as *Skierka Canarii.* The latter has smaller urediniospores and teliospores and the urediniospores have two narrow lateral wings edged with a fringe of fine teeth-like echinulations. The echinulations of *Skierka philippinensis* are arranged longitudinally in rows, the rows on the thickened band sometimes being somewhat closer and larger. The walls of the teliospores frequently show two layers, the outer separating from the inner.

SKIERKA CANARII Racib. Parasitische Algen und Pilze Javas **2**: 30. 1900.

Uredinia minute, hypophyllous, scattered or in small groups, subepidermal, covered by the epidermis except for a small pore, pulverulent; urediniospores as bounded by the inner wall obovoid, 14–16 × 24–30 μ, the inner wall hyaline or yellowish, uniform in thickness, 1–1.5 μ, the outer 1–1.5 μ, thickening into two opposite

longitudinal wings 2.5–6 μ wide, the edges provided with a fringe of curving teeth-like projections, the remainder of the spore coarsely echinulate in longitudinal lines, with the wings in the optical plane circular in outline, 24–38 $\times$ 24–30 μ (FIG. 3).

Telia minute, hypophyllous, scattered or in small groups, subepidermal, covered by the overarching epidermis, the teliospores adhering and forced out through a small pore as a short column; teliospores fusoid, 8–12 $\times$ 54–80 μ, the wall smooth, hyaline, then 1 μ or less, the apex acuminate, solid, 14–40 μ, the base truncate (FIG. 11).

SPECIMENS EXAMINED:

Canarium commune L., Buitenzorg, Java, 1900, M. Raciborski, Sydow Ured. *2289*.

Canarium moluccanum Blume, Purmaredjo, Java, Aug. 1905, S. H. Koorders.

Canarium sp. Sattelberg, Morobe, New Guinea, March 3, 1936, Clemens, *1924*.

Koorders (**10**) has also reported this species from Java. The specimens reported by Arthur and Cummins (**4**) prove to be the preceding species. The Sydows (**16**) and Sydow and Petrak (**18**, **19**) have reported *S. Canarii* on *Canarium villosum* for the Philippines. It is probable that some at least of the collections are *S. philippinensis*.

The lateral wings of the urediniospores are best seen when in the optical plane. They are lateral enlargements of the outer hyaline wall usually vertical, sometimes somewhat oblique. The fringe of slender, curving teeth on their margins is one of the distinguishing marks of the species. This and the small urediniospores separate this species from *S. philippinensis*.

The teliospores adhere somewhat and are forced through the small opening of the telium as a column. They are not catenulate but as Raciborski has pointed out, the younger spores are forced up between the older.

Skierka cristata (Speg.) comb. nov.

Uredo cristata Speg. Anal. Soc. Ci. Argent. **17**: 119. 1884.

Uromyces Cupaniae Arth. & Johnston, Mem. Torrey Club. **17**: 131. 1918.

Ctenoderma cristatum Sydow, Ann. Myc. **17**: 103. 1920.

Pycnia amphigenous, grouped in hypertrophied areas 2–5 mm. across, subepidermal, oblate-spheroid, 100–200 μ wide, 80–100 μ thick, ostiolar filaments present.

Uredinia mostly hypophyllous, deep seated in the hypertrophied tissue, covered by a thin layer of compacted hyphae beneath the epidermis, opening by a small pore, pulverulent; urediniospores as bounded by the inner wall narrowly obovoid or fusoid, 16–20 $\times$ 30–40 μ, the inner wall yellowish, uniform in thickness, 1.5–2.5 μ, the outer wall hyaline, swelling to form a longitudinal plate, the wings reaching 10–15 μ in width over the upper portion of the spore, with the plate in the optical plane the spores obovate or fusiform in outline, 22–30 $\times$ 40–55 μ, coarsely and sparsely echinulate in the upper portion, crenate or serrate in lines on the edges of the lateral wings, the pores obscure, the apices rounded or acute (FIG. 4).

Telia hypophyllous, scattered or in small groups, similar to the uredinia, the spores adhering and often forced out in long, delicate, white threads; teliospores fusoid, 10–15 $\times$ 60–96 μ, the wall thin, 1 μ, of two layers, the outer finally separating from the inner, the apex acuminate, the base truncate (FIG. 12).

SPECIMENS EXAMINED:

Cupania americana L. Maravale Valley, Port-of-Spain, Trinidad, May 14, 1913, R. Thaxter *40*, Rel. Farl. *673, III.*

Cupania belizensis Standl., Benque Viejo, El Cayo, British Honduras, July 17, 1936, E. B. Mains, *3524, III.*

Cupania macrophylla A. Rich. San Antonio de las Baños, Cuba, June 11, 1916, J. R. Johnston, *779, O II III*; Taco Taco, Cuba, Sept. 17, 1916, J. R. Johnston, *876, O II*; Santiago de las Vegas, Cuba, Dec. 3, 1916, J. R. Johnston, *927, O II.*

Cupania sp. Santiago de las Vegas, Cuba, June 6, 1905, C. F. *Baker, 88, O II III*; Paraguay, Jan. 1882, Speg. F. Guar. *131.*

Arthur and Johnston (**5**) decided that the urediniospores of this rust were teliospores and placed it in the genus *Uromyces* under the name *Uromyces Cupaniae* since the name *Uromyces cristata* was preoccupied. They noted a similarity to *Skierka.* The Sydows (**17**) also concluded that the urediniospores were teliospores and proposed the genus *Ctenoderma* with this as the type species. However, teliospores typical of the genus *Skierka* occur abundantly on two specimens (Johnston, *779* and Baker, *88*) closely associated with the sori interpreted as telia by Arthur and Johnston and the

Sydows. It is evident that the latter are uredinia. In addition to the specimen from British Honduras, Thaxter's collection (Rel. Farl. *673*) from Trinidad bears telia (Arthur, *2*). As in other species of *Skierka* the walls of the urediniospores are laterally thickened, more so than in the previous species. The outer wall swells in water. This takes place unequally and forms a thick plate surrounding the spore longitudinally except for the hilum. The edges of the plate are crenate or serrate giving the spore its cristate appearance. The teliospores are very similar to those of other species of *Skierka*. They adhere and are forced through the narrow openings of the telia as long delicate white threads. The wall of the teliospore separates into two layers. The long accuminate apex of the teliospore is solid for most of its length. When the teliospore germinates the wall breaks and the solid apex is pushed off.

Skierka Diploglottidis (Cooke & Massee) comb. nov.

Uromyces Diploglottidis Cooke & Massee, Grevillea **17**: 55. 1889.

Ctenoderma Diploglottidis Sydow, Ann. Myc. **20**: 55. 1922.

Uredinia mostly epiphyllous, in green islands, subepidermal, covered by the epidermis and a thick (10–20 μ) compacted layer of hyphae except for a small pore or slit; urediniospores as bounded by the inner wall, oblong-fusoid, 12–16 $\times$ 32–42 μ, the inner wall yellowish, 1.5–3 μ thick, the outer hyaline, thickened to form two opposite longitudinal lateral plates, with the plates in the optical plane, the spores elliptic-fusiform in outline, 22–28 $\times$ 40–60 μ, the apices acute, the edges of the plates crenate (FIG. 5).

Telia similar to the uredinia; teliospores in the specimen examined collapsed, apparently fusoid, 15–18 $\times$ 70–90 μ, the wall colorless, 1.5 μ, the apex acute.

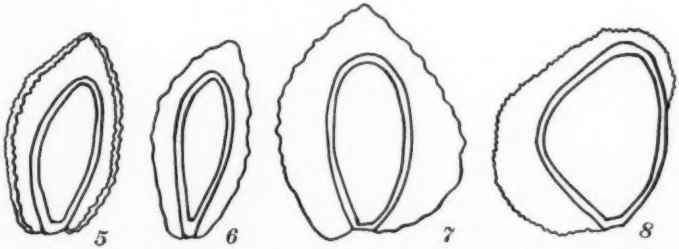
SPECIMEN EXAMINED:

Diploglottis sp. Brisbane, Queensland, Australia, Bailey, *626*, type.

Cooke (**6**) described the urediniospores as teliospores. Sydow (**13**) studied the specimen in the Kew Herbarium and decided that the rust was a species of *Ctenoderma*. He gives the host as

Diploglottis Cunninghamii. Sydow (**15**) has reported another collection from Australia.

The type specimen apparently has teliospores characteristic of *Skierka.* They are collapsed and are difficult to study. The



FIGS. 5–8. Urediniospores of species of *Skierka* and *Ctenoderma* with lateral thickenings of the wall in the optical plane, × 600: 5, *S. Diploglottidis;* 6, *S. Petchii;* 7, *S. Holwayi;* 8, *Ctenoderma Toddaliae.*

spores which have been described as teliospores are apparently urediniospores similar to those of other species of *Skierka* specially *S. cristata,* having somewhat similar lateral thickenings of the wall.

Skierka Petchii (Sydow) comb. nov.

Ctenoderma Petchii Sydow, Ann. Myc. **21**: 342. 1923.

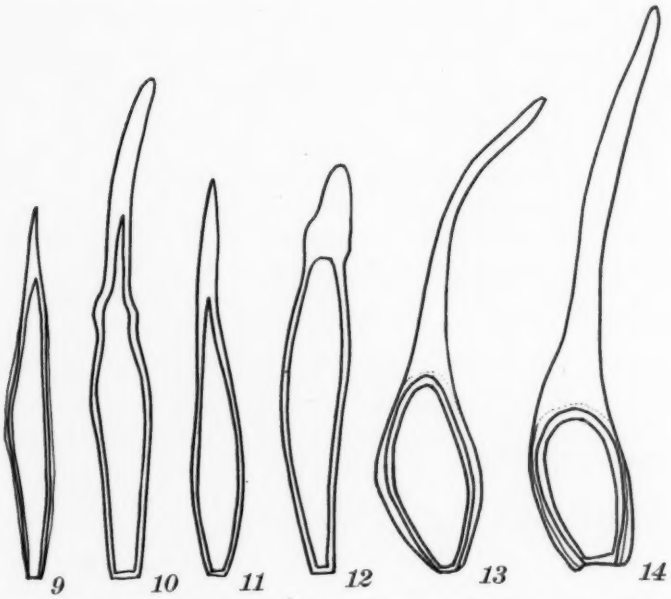
Uredinia amphigenous, crowded in reddish-brown spots, subepidermal, covered by the epidermis except for a small pore or slit; urediniospores narrowly ellipsoid as bounded by the inner wall, 8–12 × 24–50 μ, the inner wall yellowish, 1.5–2 μ thick, the outer wall hyaline, thickened to form two lateral longitudinal wings, with the wings in the optical plane, the urediniospores fusiform in outline, 18–20 × 39–60 μ, the apices acute, the edges of the wings irregularly crenate (FIG. 6).

Telia similar to the uredinia, the teliospores adhering to form short columns; teliospores obovoid-fusoid, excluding the apex, 12–18 × 38–44 μ, the apex long attenuate, 25–50 μ long, the wall hyaline, the inner 1 μ, the outer thinner and separating from the inner (FIG. 13).

SPECIMENS EXAMINED:

Sapindus bifoliatus Hiern, Sigiriya, Ceylon, Aug. 1912, T. Petch, *3565,* type.

According to Sydow (**14**), Petch reported this collection as *Uredo cristata* Speg. Sydow (**14**) described it as a species in *Ctenoderma,* the urediniospores being described as teliospores. A few telia have been found accompanying the uredinia. These



FIGS. 9–14. Teliospores of species of *Skierka,* × 600: 9, *S. congonensis;* 10, *S. philippinensis;* 11, *S. Canarii;* 12, *S. cristata;* 13, *S. Petchii;* 14, *S. Holwayi.*

contain teliospores typical of the genus *Skierka.* The urediniospores also are characteristic of that genus. The species is nearer *S. Holwayi* than *S. cristata.* The long attenuate apices of the teliospores and the lateral thickenings of the wall of the urediniospores approach those of the former species.

SKIERKA HOLWAYI Arth. Am. Jour. Bot. **5**: 433. 1918.

Pycnia amphigenous, in small groups, subepidermal, discoid, 350–450 μ wide, 90–130 μ thick.

Primary uredinia mostly epiphyllous, in groups surrounding the

pycnia, developing immediately beneath the greatly enlarged epidermal cells, flask-shaped, covered by a layer of compacted hyphae beneath the epidermis, opening by a pore, the urediniospores sometimes emerging in sporehorns, which easily disintegrate in water. Secondary uredinia rare, scattered, subepidermal, the epidermal cells not enlarged; urediniospores as bounded by the inner wall narrowly obovoid, 14–20 × 30–65 μ, the inner wall yellowish-brown, uniform in thickness, 2–2.5 μ, the outer wall hyaline, swelling laterally to form a plate reaching 26–36 μ in width longitudinally surrounding the spore except for the hilum, with this plate in the optical plane the spores ovate or cordate in outline, 26–36 × 30–65 μ, acute at the apex, appearing smooth except for the edges of the plate which are irregularly crenate (FIG. 7).

Telia, mostly hypophyllous, accompany the pycnia and primary uredinia and then similar to the latter, sometimes scattered and then not causing enlargement of the epidermal cells, the teliospores adhering and forming long yellowish columns; teliospores fusoid, 11–14 × 28–38 μ exclusive of the apex which is very slender, reaching a length of 60 μ, the apex disintegrating in water, the wall smooth, hyaline, of two layers, the inner 1.5 μ, the outer 1 μ or less and separating from the inner (FIG. 14).

SPECIMENS STUDIED:

Thouinidium decandrum (H. B. K.) Radlk. Sanarate, Guatemala, Feb. 10, 1916, E. W. D. Holway, *475;* Amapala, Isla Tigre, Honduras, Feb. 14, 1922, Paul C. Standley, *20730a;* San Miguel, El Salvador, Feb. 24–27, 1922, Paul C. Standley, *21081.*

Thouinidium sp. Agua Caliente, Guatemala, E. W. D. Holway, *849* (type).

This is a most unusual rust. In this species many of the characters stressed in the other species find their extreme development. The outer wall of the urediniospores swells unequally in water to form a broad and thick plate surrounding the spore longitudinally except for the hilum. This extension of the wall is so broad that in mounts for the microscope the spores nearly always orient themselves with the plate in the optical plane and the spore can be rotated with difficulty. The urediniospores sometimes adhere and emerge from the uredinia in sporehorns. They, however, readily separate in water. The teliospores adhere and are forced through the narrow opening of the telium in long columns. They separate less readily than in other species. The teliospores have very long filiform apices and the wall of the spore separates

into two layers. In water the apex disintegrates. This leaves the outer layer free from the inner except at the base of the spore.

SKIERKA AGALLOCHOA Racib. Bull. Acad. Sci. Cracovie. 1909: 275.

Specimens of this species have not been available. It is apparently known only from the original collection obtained at Batavia, Java on *Excoecaria Agallocha*. Only telia are described. The teliospores are given as 8–12 × 60–100 μ, smooth, thin-walled, the apex 18–25 μ. The teliospores are forced through the opening of the telium in threads 50–80 μ wide and 1–8 mm. long.

SKIERKA ROBUSTA Doidge, Bothalia **2**: 155–156. 1926.

Specimens of this species have not been available. Only telia are described on *Rhoicissus rhomboidea* from South Africa. The teliospores are given as yellowish, narrow-lanceolate or lanceolate-fusiform, 20–27 × 120–180 μ, acuminate, elongated into a long filiform process, the wall 3–3.5 (5) μ.

CTENODERMA TODDALIAE (Petch) Sydow, Ann. Myc. **17**: 103. 1919.

Aecidium Toddaliae Petch, Ann. Roy. Bot. Gard. Peradeniya **4**: 303. 1909.

Uredo Toddaliae Petch in Sydow, Fungi exotici exsiccati *69*. 1913.

Pycnia amphigenous, grouped in yellowish spots, discoid, 150–200 μ wide, 50–75 μ thick, filaments lacking.

Uredinia hypophyllous, surrounding the pycnia, deep seated in the host tissue, opening by a small pore or slit; urediniospores very angular, 28–36 × 40–70 μ, the inner wall yellowish-brown, uniform, 1.5 μ, the outer wall hyaline, very thin over most of the spore, thickened irregularly to form ridges of various extents mostly over the apex or at the base, occasionally from base to apex, up to 10 μ thick, irregularly and finely echinulate, specially on the ridges (FIG. 8).

SPECIMENS EXAMINED:

Toddalia aculeata, Hakgala, Ceylon, Aug. 30, 1912, T. Petch, Sydow, Fungi exot. *69*.

Only pycnia and uredinia were found on the specimen studied. This is probably a species of *Skierka*. The urediniospores have the outer wall thickened in ridges which are less regular than in the other species. Until telia are discovered it seems best to leave this species under the name *Ctenoderma Toddaliae*.

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EXPLANATION OF FIGURES

FIG. 1–4. Urediniospores of species of *Skierka* with lateral thickenings of the wall in the optical plane, × 600: 1, *Skierka congonensis;* 2, *S. philippinensis;* 3, *S. Canarii;* 4, *S. cristata.*

FIG. 5–8. Urediniospores of species of *Skierka* and *Ctenoderma* with lateral thickenings of the wall in the optical plane, × 600: 5, *S. Diploglottidis;* 6, *S. Petchii;* 7, *S. Holwayi;* 8, *Ctenoderma Toddaliae.*

FIG. 9–14. Teliospores of species of *Skierka,* × 600: 9, *S. congonensis;* 10, *S. philippinensis;* 11, *S. Canarii;* 12, *S. cristata;* 13, *S. Petchii;* 14, *S. Holwayi.*

STUDIES ON HISTOPLASMA CAPSULATUM AND SIMILAR FORM SPECIES—I. MORPHOLOGY AND DEVELOPMENT [1]

ARDEN HOWELL, JR.[2]

(WITH 5 FIGURES)

INTRODUCTION

The identity of the organism which is the etiological agent of the disease in man known as histoplasmosis has long been in question. The disease was originally described by Darling (**4, 5, 6**) as a result of his studies among the natives of Panama when first he encountered a case characterized clinically by emaciation, splenomegaly, leucopenia, anemia, and irregular pyrexia. Subsequently an autopsy upon the patient revealed that the pathological features were as follows: "The invasion of the endothelial cells in the smaller lymph and blood vessels and capillaries by enormous numbers of a small, encapsulated micro-organism causing necroses of the liver with cirrhosis, splenomegaly, pseudo-granulomata of the lungs, small and large intestines, with ulceration of the latter, and necrosis of the lymph nodes draining the infected viscera."

The organism, which Darling regarded as the cause of the disease that produced this condition, occurred in the tissues as small, round to oval cells, 1–4 μ in diameter, some of which Darling thought possessed flagella. Although he admitted these forms resembled the Leishman-Donovan bodies of kala-azar they differed from the latter in several respects, and hence were regarded by

[1] Contribution No. 163 from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University.

[2] The author is greatly indebted to Dr. David H. Linder, under whose direction the present investigations were conducted, for the material he provided to begin the work, for his many valuable suggestions, assistance, advice, and encouragement, and for the manner in which he gave so unstintingly of his time. Also he wishes to express his gratitude to Dr. William H. Weston, Jr., for his constructive criticism and for his helpful cooperation throughout the work.

Darling as a hitherto undescribed protozoön parasite to which he gave the name *Histoplasma capsulatum*. Two additional cases of this disease were observed by Darling within the next few months, but all attempts to cultivate the organism failed.

Following Darling, the disease was reported by Riley and Watson (**17**), and Crumrine and Kessel (**3**) from Minnesota and California respectively. It was not until 1934 that the first successful attempt to grow the pathogen in artificial culture was made by de Monbreun (**7**) who isolated the organism from the spleen of an infant that had died of the disease in 1932 in Tennessee. The results of his study showed the parasite to be of fungal nature and that the life history was divided into two phases, a parasitic, yeast-like phase which germinated to produce the saprophytic, mycelial phase in culture. The only means found thus far to reconvert the mycelial form into the yeast-like phase has been to inoculate the mycelial form into susceptible animals. From the lesions so produced, the yeast-like form has been obtained on suitable culture media.

In an attempt to place this organism in a system of classification de Monbreun compared it with such forms as *Endomyces capsulatus* Rewbridge, Dodge, and Ayers (**16**). This fungus was isolated from a nodule on the surface of the medulla in a case which had been diagnosed as meningoencephalitis with complicating tuberculosis. In the lesions this *Endomyces* occurred exclusively as budding cells, but on artificial culture media it developed a mycelial form quite similar to that described for *Histoplasma*. However, de Monbreun made no definite attempt to classify *H. capsulatum* other than to point out the similarities between its life cycle and cultural characteristics and those of *Endomyces*.

Later Hansmann and Schenken (**9**) isolated a fungus from a patient suffering from a generalized skin infection very similar to histoplasmosis. The authors considered this fungus was not identical with *Histoplasma capsulatum* but was rather a pathogenic species referred tentatively to the genus *Sepedonium*.

That the fungus described by Hansmann and Schenken (**9**) as a parasitic *Sepedonium* was identical, or nearly so, with *Histoplasma capsulatum* Darling has been established by Moore (**15**) and others. Since, however, Moore erroneously considered the

large, tuberculate, ascus-like bodies mentioned by de Monbreun (**7**) as occurring on the mycelia produced by these two organisms on artificial culture media to be true asci containing endospores, he transferred these fungi to the genus *Posadasia*. Inasmuch as Moore also observed certain cultural and morphological differences between the two strains, he created the species *Posadasia pyriformis* Moore for the fungus isolated by Hansmann and Schenken, and *Posadasia capsulata* (Darling) Moore for the form first cultured by de Monbreun (**7**).

The genus *Posadasia* was established by Canton (**1**) for the organism which was the etiological agent of coccidioidal granuloma in South America. In the tissues this parasite usually exists as large, thick-walled cells which are transformed directly into asci containing an indefinite number of ellipsoidal spores. Moore (**14, 15**) noticed the resemblance of these asci to the ascus-like bodies (or so-called "asci") produced on the mycelium of *Histoplasma* on artificial culture media, and presumed that they were homologous structures. It was on this basis that Moore made the generic transfer.

On the other hand, Dodge (**8**) reduced the genus *Posadasia* to synonomy with the genus *Coccidioides* Stiles, the latter having been created for the organism which was the etiologic agent of coccidioidal granuloma in North America, inasmuch as he, Dodge, believed the two parasites which had been described as the causative agents of this disease were identical. He then retained the name *Histoplasma capsulatum* for the organism described by Darling (**4**), making *Posadasia capsulata* (Darling) Moore a synonym of this form, and likewise created the new combination *Histoplasma pyriforme* (Moore) Dodge for the organism described by Moore from the case of histoplasmosis isolated by Hansmann and Schenken. He then placed the genus *Histoplasma* in the family Coccidioideaceae of the Endomycetales with the genera *Coccidioides, Paracoccidioides,* and *Rhinosporidium*. Each of these produce similar lesions in man, are more or less similar in their parasitic phases and are similar in culture, inasmuch as each, in culture, produces structures that have been regarded as naked, multi-spored asci. According to Moore (**15**) and Dodge (**8**) these genera differ in that the "asci" in *Coccidioides* are

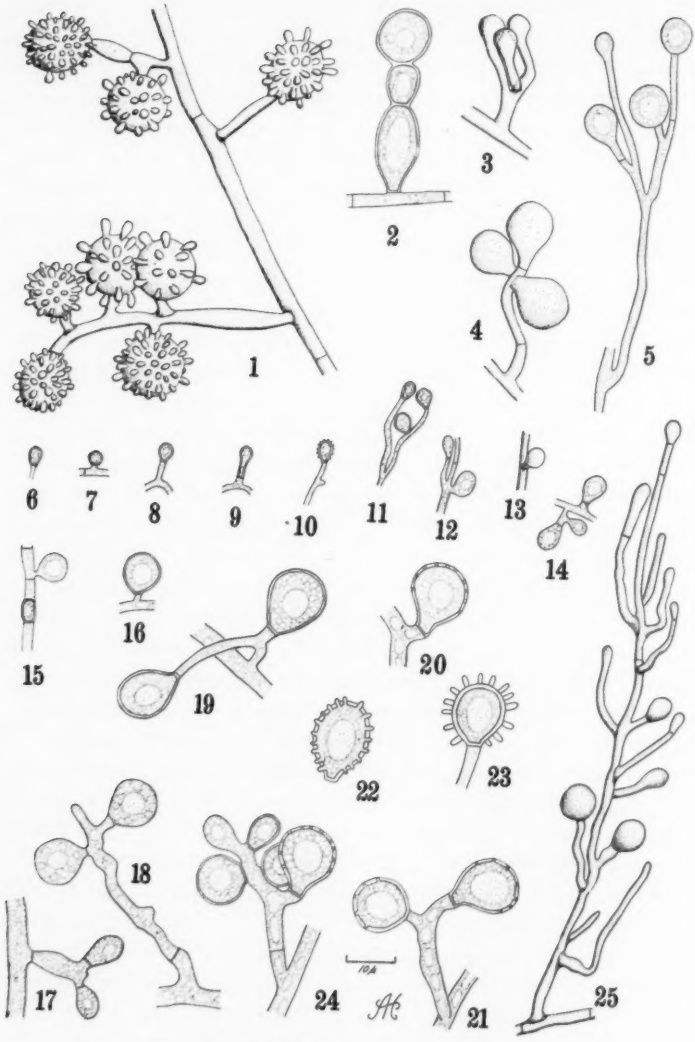

FIG. *A. Histoplasma capsulatum* Darling.

smooth-walled; in *Histoplasma* bear functionless tubercles on the walls; in *Paracoccidioides* bear tubercles into which the "ascospores" migrate and from which they are then discharged by the rupture of the walls; while in *Rhinosporidium* the "asci" open by a definite pore.

In a recent paper Ciferri and Redaelli (2), after making a careful study of *Histoplasma capsulatum* Darling and *H. pyriforme* (Moore) Dodge, reduced the latter species to synonomy with *H. capsulatum* because of the fact that the two species are identical morphologically. They, therefore, listed the following synonomy:

HISTOPLASMA CAPSULATUM Darling, 1906.

Cryptococcus capsulatus Neveu Lemaire, 1921.
Posadasia capsulata Moore, 1934.
Posadasia pyriformis Moore, 1934.
Sepedonium sp. Hansmann & Schenken, 1934.
Histoplasma pyriforme (Moore) Ciferri & Redaelli, July, 1935.
Histoplasma pyriforme (Moore) Dodge, August, 1935.

Furthermore, they placed the genus *Histoplasma* in the new family Histoplasmaceae which they included in a super-family of non-sporogenous yeasts, the *Adelosaccharomycetaceae* Guill. as emended by Ciferri (1930).

It is readily seen that much confusion exists as to the exact identity and classification of the organism which is the etiological agent of Darling's histoplasmosis. It is the aim of this paper, therefore, to determine its true identity and taxonomic position, first, by presenting a detailed account of its life history and development with ample descriptions and illustrations, and second, by comparing these with the life cycles and spore forms of existing genera of saprophytic or parasitic fungi to which it has been referred. To supplement the present work the writer has also undertaken a comparative study of the biological reactions of representative species of these fungi in pure culture as well as an examination of the interrelationships of these species through serological studies and through experimental inoculations.

MATERIALS AND METHODS

These studies were made with pure cultures, the fungi used having been obtained from the following sources: *Histoplasma*

capsulatum Darling, *H. pyriforme* (Moore) Dodge, originally from de Monbreun (**7**) and Hansmann (**9**) respectively, deposited in the culture collection of the Cryptogamic Laboratory as *No. M250* and *No. M251; Sepedonium chrysospermum* (Bull.) Lk., *No. M283,* from Dr. D. H. Linder who isolated it from *Boletus* sp.; *S. xylogenum* Sacc., *No. M281,* and *Stephanoma tetracoccum* van Zinderen-Bakker (**18**), *No. M273,* from Dr. Linder who obtained *S. xylogenum* Sacc. from an original isolate from decayed wood by Dr. R. Thaxter and *S. tetracoccum* from an original isolation from *Geoglossum Farlowii* by Dr. Linder; and *Mycogone perniciosa* Magn., *No. M280,* and *Chlamydomyces palmarum* (Cooke) Mason, *No. M279* from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Various media, such as Sabouraud's maltose agar, four per cent sucrose (Merck) agar, one per cent Difco Bacto-peptone agar, and potato maltose agar were used for general cultural work. The latter medium, because of the fact that it was readily available, easily prepared, and proved very satisfactory for the growth of all species concerned, was chiefly used. This medium was prepared from a decoction of 100 grams of potato per liter to which was added two grams of Pfanstiehl's technical maltose and twenty-five grams of Difco Bacto-agar. To insure uniformity of the medium, four to eight liters of the decoction were made up at one time, and agar was added when needed.

Observations were usually made from Petri dish cultures or slide cultures, the latter being a modification of Unna's technique, recommended by Langeron (**10**), and essentially the same as that described by Martin et al. (**12**). In this process the fungus grows over a slide which has been placed below the surface of the agar in a Petri dish. Whenever desired, the slide is removed from the Petri dish, allowed to dry at room temperature for twenty-four to thirty-six hours, during which time the agar is dehydrated, and the slide is then stained with lactophenol cotton-blue (**11**) for 15 to 20 minutes. At the end of this time they are immersed in 70 per cent alcohol for 10 to 15 minutes, and then dehydrated and mounted in balsam.

Many observations of the fungi in Petri dishes or test tubes

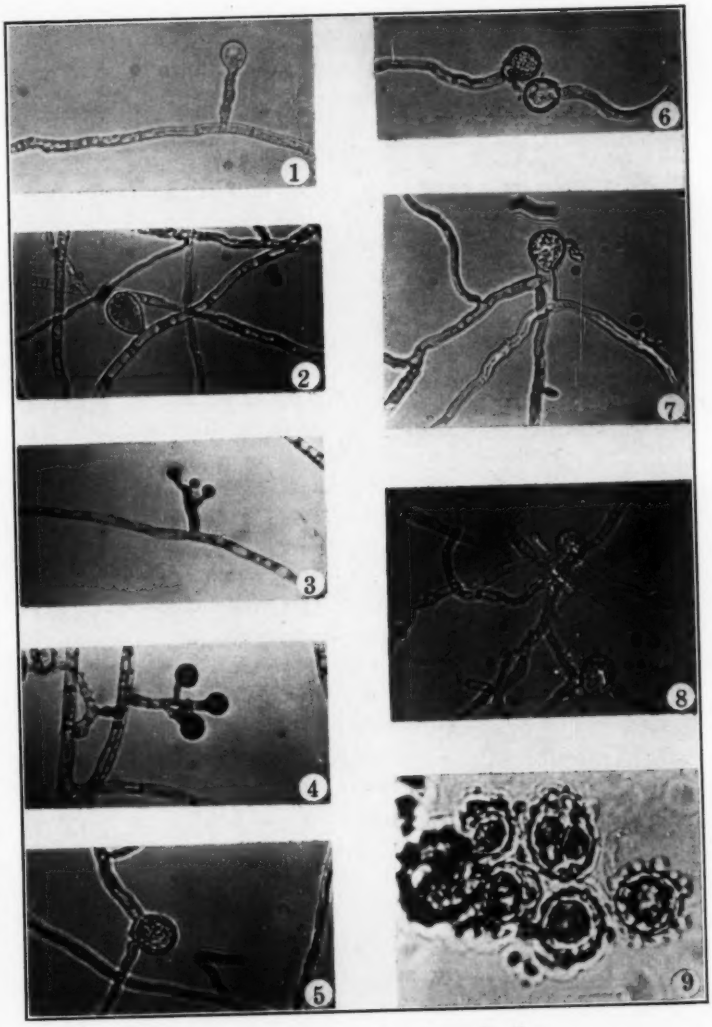

FIG. B. *Histoplasma capsulatum* Darling.

were supplemented by studies of cultures in van Tieghem cells, especially in following the germination of spores.

All cultures, unless otherwise noted, were grown at room temperature, which averages about 22° C.

LIFE HISTORIES

HISTOPLASMA CAPSULATUM Darling.

The life cycle of *Histoplasma capsulatum* Darling, like that of many of the parasitic fungi, is rather complicated, inasmuch as the habitat determines the character of the growth. As was pointed out in the introduction it exists in the tissues as small, oval, yeast-like bodies, measuring about $3 \times 3.5\ \mu$ in diameter. Each cell is surrounded by a thin membrane, and, in unstained preparations, usually contains one or more small, refractile fat droplets in addition to a single protoplasmic granule in active Brownian movement. Also, in stained preparations, there may appear one or more vacuoles in each cell, and a nucleus which usually appears as a peripheral, crescentic mass of chromatin. One pole of the cell is usually pointed, and when buds occur they do so singly and only at the pointed ends of the cells. The yeast-like form will persist, if upon isolation from the natural host, it is kept on blood or serum agars at 37° C. and transferred at short intervals. The mycelial form of the fungus develops directly from the yeast-like cells by a process of elongation to form mycelial growths which soon become septate.

The preceding description of the yeast-like phase of *Histoplasma capsulatum* has been adapted from de Monbreun's paper (**7**) since this phase has not yet been studied by the present writer whose observations follow.

The mycelium varies from 2 to $5\ \mu$ in diameter, and is usually highly refractile, branched, and multicellular, though its appearance may vary somewhat depending on the type of culture medium employed. As the mycelium matures, the walls become slightly thickened, and within the hyphae a large number of fat globules and vacuoles are formed (FIG. *B:* 1–4).

After varying lengths of time, depending on the medium employed and the conditions under which the culture develops, sporulation begins.

It would appear at first glance that there are at least three types of spores produced (FIG. *A:* 1, 6–9, 25). First, there are large, tuberculate, spherical to pear-shaped bodies or spores, 10–25 μ in diameter, sessile or on short pedicels, produced on the aerial mycelium (FIG. *A:* 1); secondly, smooth-walled, round to oval spores, varying in diameter from 5 to 20 μ, usually formed on short pedicels, and on the submerged mycelium (FIG. *A:* 2, 5, 25); and thirdly, small, smooth-walled spores, spherical to pyriform, 2–6 μ in diameter, sessile or on short stalks, produced on the aerial or submerged mycelium (FIG. *A:* 6–10).

The large, tuberculate, aerial spores begin their development as bulbous enlargements of the terminal ends of lateral branches. These branches may be simple with a single spore at the terminal end (FIG. *B:* 1; FIG. *A:* 18) or divided into two to several short branches, with a single spore developed on the terminal end of each branch or one to several are produced acropetalously and directly on a short branch (FIG. *A:* 3, 4, 17, 24; *B:* 3). As these spore initials increase in size, each one is cut off from the rest of the hyphae by a cross wall near the base, become spherical to pyriform in shape, and the wall gradually increases in thickness (FIG. *A:* 19, 24). Simultaneously, there usually develops a large central vacuole, and shortly thereafter definite softenings or pits appear in the walls (FIG. *A:* 20) through which papillate protoplasmic outgrowths protrude to form an indefinite number of finger-like processes (FIG. *A:* 21, 22). As the spores mature these papillae increase in size and are finally cut off from the protoplasmic content of the spores by the formation of a secondary inner wall (FIG. *A:* 23). When fully developed they contain numerous globules, often very uniform in size, which simulate endospores (FIG. *B:* 9). However, these globules are stained intensely by osmic acid, Sudan III and Scharlach R,[3] and disappear entirely when mounted on a slide in lactophenol and heated slightly. Furthermore, it has been observed that when they are treated with Scharlach R or Sudan III, then mounted in glycerine and allowed to stand several weeks, the globules coalesce to form

[3] Saturated alcoholic solutions of Scharlach R and Sudan III were used, prepared by dissolving 0.2 gm. of each in 100 c.c. 70 per cent alcohol; osmic acid was used in the form of vapor from a 2 per cent aqueous solution.

a single, more or less spherical droplet occupying the center of the spore. Since the osmic acid and stains mentioned are generally considered specific for fatty substances, the author regards these bodies as fat globules, and has been unable to find any evidence of true endospores within these large, tuberculate, so-called ascus-like bodies.

Inasmuch as the classification of this organism depends upon the nature of these tuberculate spores, it seems wise to consider briefly here the interpretations that have been placed upon them.

These bodies were first observed by de Monbreun (**7**), who proposed the term "ascus-like bodies or cells" as a temporary name for them since they resembled asci because of the numerous fat globules, simulating endospores, which they contain. He emphatically stated, however, that they could not be called asci since he failed to observe true endospores or any sexual phenomena in direct association with them. Likewise, Hansmann and Schenken (**9**) failed to find evidence of endospores within these tuberculate cells. They also observed the same round, hyaline bodies which simulated endospores, but they reported that these endospore-like structures absorbed fuchsin and Sudan III, disappeared upon heating, and failed to survive fixing and staining methods. Hence they considered the ascus-like cells of de Monbreun to be chlamydospores containing one to several globules of a fatty substance.

On the other hand, Moore (**15**) supposedly found true endospores within these ascus-like cells apparently in addition to the fat globules observed by de Monbreun (**7**), Hansmann and Schenken (**9**), and the present author. Moore states that "cytological investigations show that the single ascus contains a number of spherical spores which are set free by a rupture of the ascus wall to germinate and commence another cycle," but that "when in a nutrient condition, the ascus may germinate with few to several germ-tubes which develop into a mycelium." Furthermore, he figures these ascospores and their germination in plate 11, figure 1 and plate 13, figure 72 of his paper. Later in his paper, however, he refers to these same two figures as conidia which "break off easily from the hyphae and germinate to give rise to a new colony serving much the same purpose as do the

ascospores." In view of the conflicting references to plate 11, figure 1 and plate 13, figure 72 by Moore (**15**) and also because he has failed to show either fusion of nuclei or the meiotic division characteristic of the development of ascospores within an ascus, the writer feels that Moore has not shown the tuberculate structures to be asci. It is therefore impossible to accept Moore's conclusions.

The writer has repeated the technique used by Moore, insofar as it was possible to ascertain the methods used from the meager descriptions given in his paper, and has failed to find any evidence whatever for the presence of endospores within these tuberculate cells. As it has already been pointed out, there are numerous bodies, quite uniform in size, within these cells, but in view of the evidence already presented, both by the present author and previous workers with the exception of Moore, it can hardly be doubted that these bodies are fatty in nature and that there are no endospores present. Hence the structures in question cannot be considered as asci. The question then arises as to what term should be applied to these aerial, tuberculate spores. It seems clear, from the development of these as previously described in this paper (FIG. *A, B*), and from the work of others (**7, 9**) that according to the terminology proposed by Mason (**13**) they should be given the name *aleuriospores*. This name was given by Vuillemin (**19**) to certain terminal chlamydospores which are similar to conidia in color, position, size, form, and structure, but which differ from true conidia in that they are not immediately freed from the mycelium by a natural means of dehiscence.

In addition to the large, tuberculate, aerial aleuriospores there develop large, smooth-walled spores (FIG. *A:* 2, 5, 25) below the surface of the agar and minute entities (FIG. *A:* 6–10), called conidia by Moore, which are formed in old cultures and are obviously depauperate structures. That both these types are modifications of the tuberculate, normal, aerial aleuriospores there can be little doubt since a study of a large number of cultures has demonstrated that all spores not only develop in the same manner and that there is a gradual transition between them, but also they germinate in the same fashion.

Neither phialospores nor *conidia vera* have been encountered

by the author in this species, nor have any been reported by previous workers.

Germination of the aleuriospores, from a ten weeks old culture, takes place readily. Within fifteen to twenty hours after they were inoculated either into a two per cent Difco Bacto-peptone or Difco proteose peptone or potato-maltose broth well-developed germ tubes could be seen growing out from nearly all of the aleuriospores used in the preparation (FIG. *B:* 5–8). In the majority of cases there develops a single germ tube which may remain unbranched for some distance (FIG. *B:* 6) or may even branch immediately upon its emergence from the spore so that, although it would appear that there were several germ tubes, closer observation demonstrated that there was in reality only a single germ tube giving rise to one or more branches (FIG. *B:* 7). In a number of instances the germ tube enlarged into a globose structure immediately upon its emergence from the aleuriospore, and then from this there arose two to several hyphae (FIG. *B:* 8). It should be pointed out at this point that if such a structure were seen from above the spore rather than from the side it would give the impression that the aleuriospore germinated by producing many germ tubes. The author questions, consequently, if it were not such a phenomenon that Moore (**15**) observed which he has described and figured as an ascus which, in a nutrient condition, had germinated with few to several germ tubes in plate 12, figure 71 of his paper. In a few cases two separate and distinct germ tubes were seen to arise from a single aleuriospore (FIG. *B:* 5) and rarely three, but the author has never observed more than this. Whether two or three germ tubes were produced from a single aleuriospore, they always arose from the same pole.

Once the germ tube is formed, growth proceeds rapidly. The hyphae begin to branch and soon become multicellular by the formation of cross walls or septa at more or less regular intervals. As they mature, they become vacuolate and replete with fat globules. Finally, within twenty-five to forty hours after the germ tubes have emerged from aleuriospores germinated on two per cent peptone or potato-maltose broth in van Tieghem cells, sporulation begins, the aleuriospores being formed in the manner already described.

The genus *Histoplasma,* then, may be characterized as a fungus parasite in the life cycle of which there are two distinct phases. The first or parasitic phase consists of small, oval, yeast-like bodies, $3 \times 3.5\ \mu$ in diameter, which reproduce by budding, and exist primarily in the endothelial cells of the smaller lymph and blood vessels and capillaries of the host. The second or saprophytic phase, derived directly from the first, consists of a typical fungus mycelium which usually appears as a white and cottony growth in culture. The hyphae composing this mycelium vary from 2.5 to $5\ \mu$ in diameter, and bear numerous aleuriospores, either sessile or on short pedicels. These spores vary in shape from spherical to pyriform, in size from 2 to $25\ \mu$ in diameter, the majority ranging from 12 to $20\ \mu$, and contain from one to several, round to ovoid, fat globules. Their walls may vary from smooth to highly tuberculate, depending on whether the spores are produced on mycelium that is submerged, on that near the surface of the agar, or that which is strictly aerial. When tuberculate, the tubercles may vary from small, wart-like processes to long, finger-like projections, 1 to $7\ \mu$ in length.

SEPEDONIUM CHRYSOSPERMUM (Bull.) Link.

The life history and morphology of *Histoplasma* outlined above can now be compared with the genus *Sepedonium,* using *S. chrysospermum* (Bull.) Link, the type of the genus, as an example.

The mycelium of this species is composed of hyphae which vary from $2–4\ \mu$ in diameter and are usually hyaline, thin-walled, branched, and multicellular. As they mature they may become very vacuolate. When the mycelium is fairly well-developed, sporulation begins, though the time of sporulation may vary somewhat, depending on the conditions under which the culture develops.

There are at least two types of spores usually produced in culture. First, there are large, yellow to golden, warted or tuberculate, spherical aleuriospores, which begin their development as small, bulbous enlargements of the terminal ends of lateral branches of the surface or aerial mycelium. These branches may occasionally be single with a single spore at the tip, but usually divide further into a number of branches, each of which gives

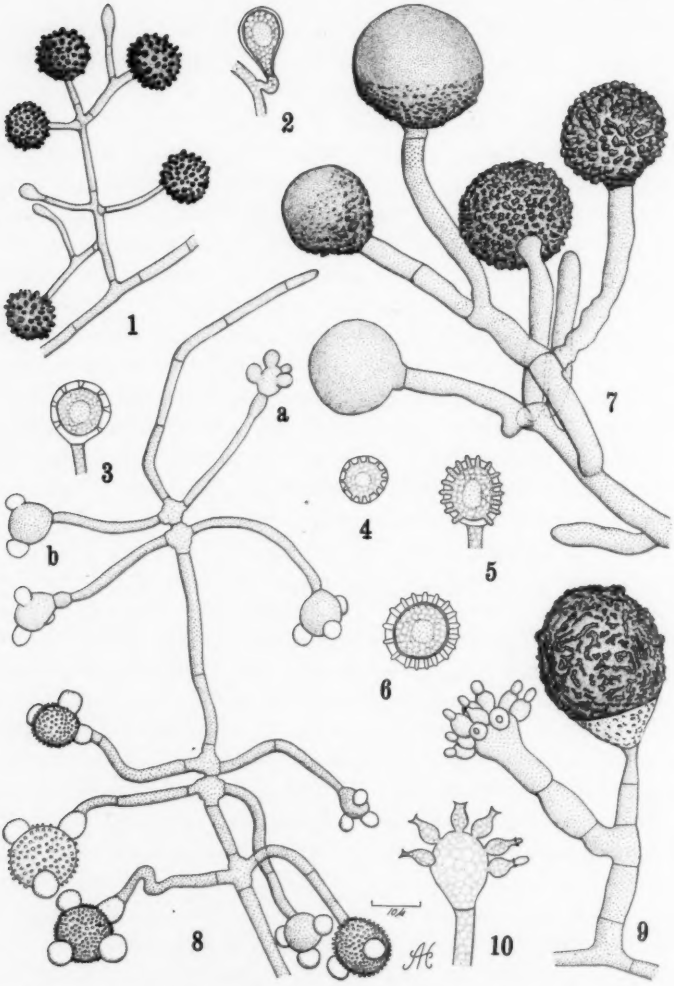

FIG. C. 1–6, *Sepedonium chrysospermum* (Bull.) Link; 7, *Sepedonium xylogenum* Sacc.; 8, *Stephanoma tetracoccum* van Zinderen-Bakker; 9, 10, *Chlamydomyces palmarum* (Cooke) Mason.

rise to a spore, so that eventually a cluster of aleuriospores is produced (FIG. *C*: 1). As these spore initials increase in size each one becomes separated from the remainder of the subtending hypha by the formation of a septum at its base, the wall increases in thickness and a large, central vacuole appears (FIG. *C*: 2). Immediately following this, definite pits or softenings in the wall make their appearance and through these protoplasmic outgrowths protrude to form short papillae (FIG. *C*: 3–5). At a slightly later stage these papillae are cut off from the protoplasmic content of the spore by the formation of a secondary inner wall (FIG. *C*: 6). As the spores continue to mature the outer wall becomes yellow to golden in color, and when fully developed the aleuriospores bear an indefinite number of these short, blunt papillae, varying from 1 to 1.5 μ in length, the spores being 14–16 μ in diameter. This process of spore formation and the production of the ornamentation is identical with that found in *Histoplasma capsulatum,* and shows these structures to be homologous in the two species. Thus additional evidence is added to substantiate the belief that the tuberculate structures of *Histoplasma capsulatum* should be thought of as aleuriospores, and not as asci.

The germination of the aleuriospores of *S. chrysospermum* is essentially similar to that already described for the homologous spores of *Histoplasma,* except that there is apparently only a single germ tube produced in *S. chrysospermum.* This process has been repeatedly observed in van Tieghem cells using either a two per cent Difco Bacto-peptone or Difco proteose peptone broth. The aleuriospores used in these studies were obtained from a five-months-old potato maltose agar culture. Within twenty to twenty-four hours after they were inoculated on the Bacto-peptone broth, short germ tubes, one from each spore, appeared. Those on the proteose-peptone broth took much longer to germinate, the germ tubes not appearing for about seventy-two hours. Once the germ tube is formed, growth proceeds rapidly, the hyphae soon becoming branched, and septate, with the branching frequently occurring at a characteristic acute angle (FIG. *E*: 3, 4). Finally, after sixty to seventy-two hours on the Bacto-peptone or after about five days on the proteose-peptone preparations, a second type of spore is produced, which, although formed before the

aleuriospores when the fungus is grown in culture, is for convenience given secondary position in this discussion. These spores, called phialospores, have not been observed by the writer, nor by any of the previous investigators, in cultures of *Histoplasma*.

The phialophore, which produces the phialospores, begins its development as a short lateral outgrowth from a hypha of the surface or aerial mycelium and by continued elongation and branching eventually forms the much-divided phialophore bearing subulate or tapering terminal cells which are known as phialides (FIG. *D:* 3). From, or within the apex of these phialides the thin-walled phialospores are abstricted (FIG. *E:* 1). Additional spores may be successively formed at the apex of a single phialide, in which process the first-formed spore is pushed aside (FIG. *E:* 2). This procedure may continue until several spores have been produced and, accumulating around the tip of the phialide, form a globose mass as a result of the adhesion of the spores. The mature phialospores are ovoid in shape, smooth-walled, hyaline, and 8–11 × 5–6 μ in size (FIG. *D:* 3). It is possible that these spores may serve an additional purpose in that they may function as spermatia in the sexual reproduction of the ascigerous stage, *Hypomyces chrysospermus* (Bull.) Tul., of which *Sepedonium chrysospermum* is the asexual or imperfect stage. The germination of the phialospores has also been observed, and is essentially similar to that of the aleuriospores, the germ tube usually arising at one pole of the spore. Occasionally a second germ tube arises at the opposite pole.

SEPEDONIUM XYLOGENUM Sacc.

For further study of the life history and morphology of *Sepedonium* in comparison with *Histoplasma* another species, *Sepedonium xylogenum* Sacc., was examined by the writer. In this form the aleuriospore has been the only type of spore developed in culture, and, so far as the writer has been able to determine, is the only spore form known. In this species the development of the aleuriospore is very similar to that of the same type of spores of *S. chrysospermum* and *Histoplasma* previously described. In addition their germination is essentially similar to

that described for *S. chrysospermum,* except that one, two, or three germ tubes may arise, whereas only one was produced in *S. chrysospermum* (FIG. *E:* 5–6). In those cases in which two or more germ tubes arise, they do so singly and at points on the spore equidistantly apart (FIG. *E:* 6).

In addition to the aleuriospores developed on the surface or aerial mycelium of *S. xylogenum,* there are small, bullate cells, resembling those of *Rhizoctonia,* though apparently not serving for vegetative reproduction, which are characteristically produced on the submerged mycelium (FIG. *E:* 7). These structures may be terminal or intercalary and are formed by a rounding up of the cells of the hyphae.

It can be seen from the preceding statements that there is a marked difference between this species and the two preceding. Aside from the lack of phialospores, the method of aleuriospore formation also distinguishes it. It may be recalled that at the beginning of the formation of papillae in those species pits or softened areas make their appearance in the walls, and then protoplasmic outgrowths protrude through the pores to form the finger-like processes. In *S. xylogenum,* on the contrary, no pits appear in the wall, but rather there is a local folding which proceeds from the base towards the apex of the spore (FIG. *C:* 7). These minute folds become thickened and eventually form the wart-like ornamentation. This difference in spore development, plus the fact that phialospores have not been observed, strongly suggests that *S. xylogenum* should be placed in some other genus. However, for the present, and since the Fungi Imperfecti are admittedly divided along artificial lines, it seems best to leave this species in the genus until it is possible to show actual connections between it and a perfect stage.

STEPHANOMA TETRACOCCUM van Zinderen-Bakker.

Another species possibly related to *Sepedonium chrysospermum* (Bull.) Link through its ascigerous stage, *Hypomyces,* is a fungus of which the conidial stage is *Stephanoma tetracoccum* van Zinderen-Bakker, parasitic on *Geoglossum.* In this species the aleuriospores begin their development as small, bulbous enlargements of the terminal ends of lateral branches of the surface or aerial

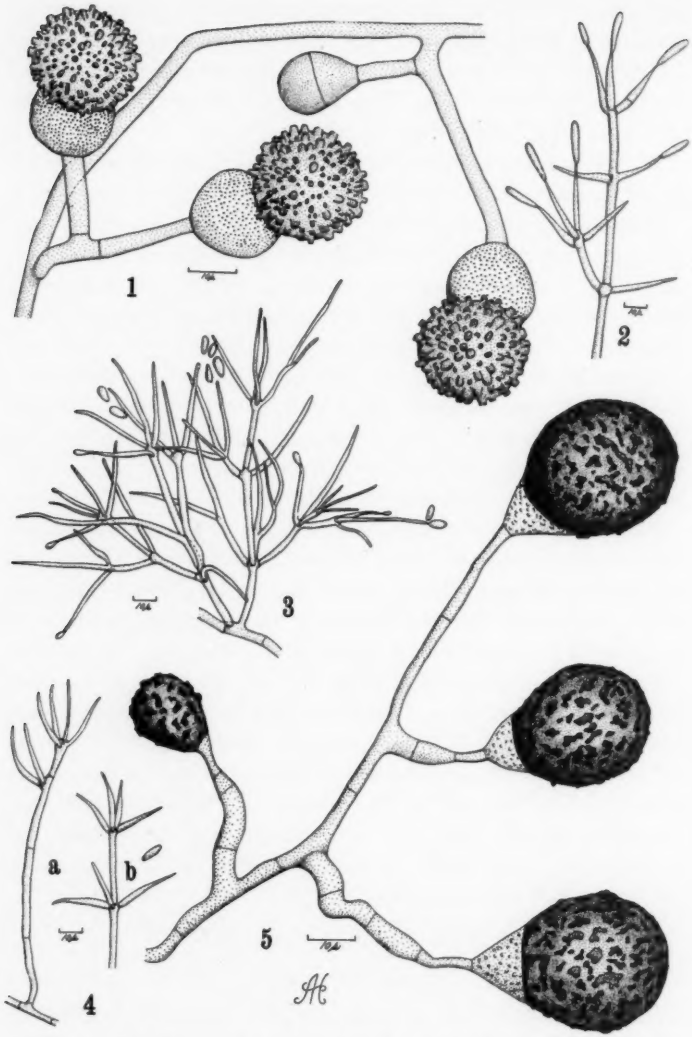

FIG. D. 1, 2, *Mycogone perniciosa* Magn.; 3, *Sepedonium chrysospermum* (Bull.) Link; 4, *Stephanoma tetracoccum* van Zinderen-Bakker; 5, *Chlamydomyces palmarum* (Cooke) Mason.

mycelium. Simultaneously, three to six globose or bullate swellings, which arise as rounded protrusions of the cell wall resulting, supposedly, from local action and pressure exerted by the protoplasm, develop on the aleuriospore initials (FIG. *C:* 8a). Shortly thereafter the spore is separated from the remainder of the hypha by a septum at its base, following which the bullate processes mentioned above are cut off from the protoplast of the spore either by septa at the bases of these swellings or by the formation of a secondary wall. Following this the protoplasmic content of the cell-like, sterile structures disappears (FIG. *C:* 8b). The remainder of the development of these aleuriospores, including the formation of the smaller echinulations, is identical with that previously described for the homologous spores of *Histoplasma* and *Sepedonium chrysospermum,* yet because of the fact that the bullate processes on the aleuriospores of *Stephanoma tetracoccum* are formed very early in their development, it differs from *Sepedonium chrysospermum.*

This species, although the fact has not been recorded by van Zinderen-Bakker (**18**), also produces in culture as well as on the natural substratum a second spore form, the phialospores (FIG. *D:* 4), which develop in the same manner as the phialospores of *Sepedonium chrysospermum* previously described.

In view of the fact that *Hypomyces Geoglossi* Ellis & Ev. has been observed in the field to be associated with this imperfect stage and also because *S. tetracoccum* produces the *Verticillium* type of phialospore, there is the strong possibility that it is related to *Sepedonium.* Since the formation of the smaller spines follows in the same manner as does that of *Sepedonium chrysospermum,* the bullate appendages need not be a source of confusion since they may be considered a secondary development.

CHLAMYDOMYCES PALMARUM (Cooke) Mason.
MYCOGONE PERNICIOSA Magn.

Two other species possibly related to *Sepedonium* through their ascigerous stages are *Chlamydomyces palmarum* (Cooke) Mason and *Mycogone perniciosa* Magn., both of which produce aleuriospores and phialospores in culture.

The aleuriospores of *Chlamydomyces palmarum* are essentially

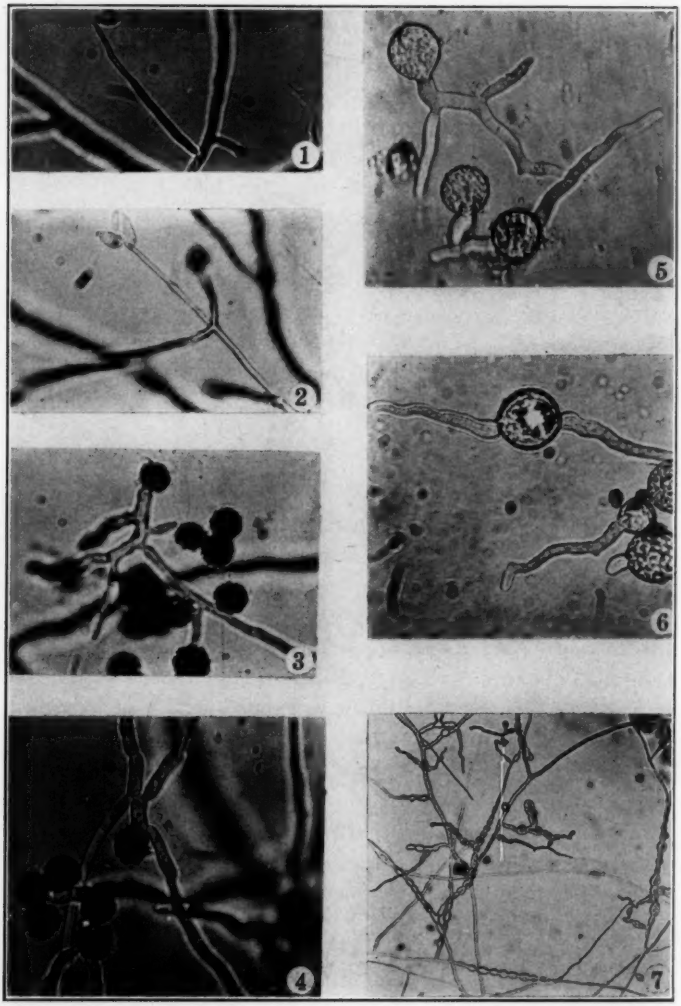

FIG. E. 1–4, *Sepedonium chrysospermum* (Bull.) Link; 5–7, *Sepedonium xylogenum* Sacc.

one-celled (the two-celled appearance being due to the enlargement of the subtending cell of the sporophore, see figure *D:* 5), large, ovoid, and tuberculate-ridged structures, subtended by a hyaline, obconic, basal cell (FIG. *C:* 9; *D:* 5). The phialophores, unlike those of the other species in which phialospores are present, are of the *Aspergillus* type (FIG. *C:* 9–10). They are irregularly swollen at the apex or they may form obovate to globose heads about 10 μ in diameter. The phialides (FIG. *C:* 9–10) are short and broad, 6–9 $\times$ 3 μ, and produce phialospores endogenously. The latter are hyaline, oblong, rounded, 4 $\times$ 2 μ, and are abstricted into mucilaginous heads or in chains.

The aleuriospores of *Mycogone perniciosa* are from the beginning two-celled structures and thus are distinct from those genera already considered. The upper cells become densely echinulate while the lower cells remain minutely warted (FIG. *D:* 1). The phialophore is similar to that produced by *Sepedonium* and *Stephanoma,* but the phialospores are more elongate and narrower in *Mycogone* (FIG. *D:* 2).

These last two genera appear to be so obviously different from *Histoplasma* and *Sepedonium* that little more need be said at this point. However, it does seem desirable to point out the general resemblances in the life histories since, because of the fact that they are related to *Sepedonium* through their congeneric perfect stages (*Hypomyces*), they will serve as controls in determining the interrelationships of these forms by future serological studies.

DISCUSSION

The organism described as a protozoön by Darling has been variously placed in classification. Its possible relationship to the Endomycetales has been indicated by de Monbreun, but on the evidence available at present, classification in this order seems premature since he did not demonstrate the presence of any ascigerous stage. Because of the presence of tuberculate spores, Hansmann and Schenken have suggested that this organism should be placed in the genus *Sepedonium.* If the manner of spore formation of *Histoplasma* and *Sepedonium* is compared, it is evident that the two genera have much in common since in both cases the

spores are formed as bulbous enlargements of the terminal ends of terminal or lateral branches. However, *Histoplasma* differs from *Sepedonium* in that during its life cycle the former not only produces yeast-like bodies in its parasitic phase but also lacks phialospores during its saphophytic phase.

The classification of *Histoplasma* has been further confused by Moore as a result of his misinterpretation of the aleuriospores which he considered to be true asci, evidence for which statement is wholly untenable especially since he has presented no cytological evidence of nuclear fusion or of meiotic division and the work of de Monbreun, substantiated by the writer, has shown that the so-called ascospores are merely fatty globules which stain readily with Sudan III, Scharlach R, and osmic acid, and furthermore, may be made to disappear by suitable reagents or by heating.

Therefore, since the ascigerous stage has not been demonstrated and also, since the spores formed in *Histoplasma* are homologous with those of *Sepedonium*, that is, are aleuriospores, it would seem advisable for the present to consider *Histoplasma* as a separate genus in the Fungi Imperfecti that shows a form relation to *Sepedonium*, *Stephanoma*, and the related genera discussed in this paper. It is hoped in a subsequent paper that the actual relationships of these species may be demonstrated and as a result the classification of *Histoplasma* may be determined.

SUMMARY

1. *Histoplasma*, *Sepedonium*, *Stephanoma*, *Chlamydomyces* and *Mycogone* have been studied in culture.

2. The life histories and morphological development of these fungi have been compared; the so-called asci of *Histoplasma* have been shown to be aleuriospores.

3. It has been proven by culture methods that *Stephanoma tetracoccum* van Zinderen-Bakker produces a phialospore stage and has been suggested that *S. tetracoccum* is the imperfect stage of *Hypomyces Geoglossi* Ellis & Ev.

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EXPLANATION OF FIGURES

The drawings were made with the aid of a camera lucida and for the most part from material mounted in lacto-phenol cotton-blue. With but few exceptions, noted in the explanation of the figure, all drawings were made at a magnification of approximately 2150. The present figures are reduced to a magnification of 750. For convenience an absolute scale representing ten microns is included in each figure.

FIG. *A*. 1–25. *Histoplasma capsulatum* Darling.

1. Portion of aerial mycelium to show arrangement of mature aleuriospores. Typical growth on 2 per cent sucrose (Merck), 1 per cent Difco Bacto-peptone agar after 7 weeks. 750 ×.

2. Optical section of a developing aleuriospore; an abnormal type of development. After 66 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell. 750 ×.

3–4. Cluster of aerial aleuriospores (sessile in 4) in an early stage of development. After 66 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell. 750 ×.

5. Smooth-walled type of aleuriospores developed below surface of agar. After 6 weeks on 4 per cent sucrose (Merck) agar. 750 ×.

6–10. Optical sections of small, mature aleuriospores developed below surface of agar. After 7 weeks on sucrose (Merck) agar. Note that in 10 the small spores may be echinulate as well as smooth. 750 ×.

11–16. Aleuriospores showing variations in size from the small type, arrested in development, to the large, normal type, all produced on the submerged mycelium. 7 weeks on 4 per cent sucrose (Merck) agar. 750 ×.

17–23. Successive stages in development of the tuberculate aleuriospores, all drawings shown in optical section. After 66 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell. 750 ×.

24. Optical section through a cluster of aerial aleuriospores showing their acropetalous development. 750 ×.

25. Successive stages in formation of the smooth-walled aleuriospores formed below the surface of agar; after 6 weeks on 4 per cent sucrose (Merck) agar. 750 ×.

FIG. *B*. The photomicrographs were taken from material in van Tieghem cell preparations at a magnification of 600. The present figures are reduced to a magnification of approximately 400.

1–9. *Histoplasma capsulatum* Darling.

1. Early stage in formation of pedicellate aleuriospores on aerial mycelium. After 70 hours on potato maltose broth in a van Tieghem cell.

2. Early stage in formation of a sessile aleuriospore on submerged mycelium. After 70 hours on potato maltose broth in a van Tieghem cell.

3. Early stage in development of cluster of aerial aleuriospores produced acropetalously on a short lateral branch. After 66 hours on 2 per cent Difco proteose peptone in a van Tieghem cell.

4. Cluster of mature aerial aleuriospores showing moderately tuberculate wall. After 66 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell.

5. Germination of aleuriospore showing two germ tubes, both arising at

the same pole of the spore. After 15 hours on 2 per cent Difco proteose peptone in a van Tieghem cell.

6. Germination of aleuriospores showing single germ tubes. After 15 hours on 2 per cent Difco proteose peptone in a van Tieghem cell.

7. Germination of an aleuriospore showing a single germ tube which branches shortly after its emergence from the spore. After 15 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell.

8. Germination of an aleuriospore showing enlargement of a single germ tube into a globose structure, immediately upon its emergence from the spore, from which arise several hyphae. After 26 hours on potato maltose broth in a van Tieghem cell.

9. Aerial aleuriospores from 7 weeks old culture on 1 per cent Difco Bacto-peptone, 2 per cent sucrose (Merck) agar, showing fat globules stained 12 hrs. with Sudan III. 800 ×.

FIG. *C*. 1–6. *Sepedonium chrysospermum* (Bull.) Link.

1. Cluster of aleuriospores showing spore initials and mature spores. After 2 weeks on potato maltose agar. 645 ×.

2–6. Successive stages in the formation of aleuriospores to illustrate the manner of formation of the spines and the close parallel existing between it and that of *Histoplasma capsulatum*. 750 ×.

7. *Sepedonium xylogenum* Sacc. Cluster of aleuriospores. After 8 days on potato maltose agar. 750 ×.

8. *Stephanoma tetracoccum* van Zinderen-Bakker. Mature aleuriospores and stages in their formation at the end of 6 weeks on potato maltose agar. 750 ×.

9–10. *Chlamydomyces palmarum* (Cooke) Mason.

9. Single aleuriospore and phialophore showing phialides and immature spores. From 1 week old culture on potato maltose agar. 750 ×.

10. Longitudinal section of phialophore showing phialides and formation of phialospores. 750 ×.

FIG. *D*. 1–2. *Mycogone perniciosa* Magn.

1. Aleuriospores, showing their arrangement and formation. After 8 days on potato maltose agar. 750 ×.

2. Single phialophore, showing phialides and phialospores. After 8 days on potato maltose agar. 400 ×.

3. *Sepedonium chrysospermum* (Bull.) Link. Single phialophore, showing phialides and phialospores. After 4 weeks on 2 per cent Difco Bacto-peptone broth in a van Tieghem cell. 360 ×.

4*a* and *b*. *Stephanoma tetracoccum* van Zinderen-Bakker. Two phialospores showing phialides, and a phialospore isolated from *Geoglossum* sp. 360 ×.

5. *Chlamydomyces palmarum* (Cooke) Mason. Cluster of aleuriospores. 750 ×.

FIG. *E*. 1–4. *Sepedonium chrysospermum* (Bull.) Link.

1. Tip of phialide showing single phialospore. 46 hours after aleuriospores had been inoculated on 2 per cent Difco Bacto-peptone broth in a van Tieghem cell. 360 ×.

2. Tip of phialide showing a cluster of phialospores. 46 hours after aleuriospores had been inoculated on 2 per cent Difco Bacto-peptone broth in a van Tieghem cell. 360 ×.

3–4. Germination of aleuriospores. After 46 hours on 2 per cent Difco Bacto-peptone broth in a van Tieghem cell. 360 ×.

5–7. *Sepedonium xylogenum* Sacc.

5–6. Germination of aleuriospores. After 12 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell. 360 ×.

7. Bullate bodies produced on submerged mycelium. After 36 hours on 2 per cent Difco proteose peptone broth in a van Tieghem cell. 320 ×.

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A STUDY OF BOTRYOSPHAERIA RIBIS ON WILLOW

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(WITH 19 FIGURES)

INTRODUCTION

Botryosphaeria Ribis was first described in 1911 by Grossenbacher and Duggar (**2**) as the cause of cane blight of species of *Ribes*. This fungus was not reported to attack other species of plants until 1924, when it was collected on *Aesculus hippocastaneum* L. and *Rosa setipoda* Hemsl. and Nils. (**8**). Subsequent investigations by Shear, Stevens, and Wilcox (**6**), Stevens (**10**), Stevens and Shear (**9**), Reichert and Hellinger (**4**), Savastano (**5**), and Smith (**7**) have extended the host range to include woody species in nearly a score of families and have shown that the organism has a wide geographic distribution.

Although Shear [1] in 1916 collected *B. Ribis* on willow at Arlington, Virginia, the first account of its occurrence on the genus *Salix* is that of Stevens (**10**), in 1924, from collections made in four localities in Florida. *B. Ribis* has subsequently been collected on pussy willow, *Salix discolor* Muhl., in Arkansas, Georgia, Mississippi, North Carolina and Virginia. Although the organism is apparently widely prevalent on species of *Salix* throughout the southeastern United States no studies have as yet been made of the morphology and development of *B. Ribis* in connection with willow canker.

APPEARANCE OF THE DISEASE

The first evidence of infection of willow trees by the fungus is afforded by the presence of occasional blighted twigs and branches. At any time during the growing season, the leaves on an infected

[1] A record of collections of *B. Ribis* on willow in the Office of Mycology and Disease Survey, U. S. Department of Agriculture, was supplied by Dr. H. A. Edson for which courtesy grateful acknowledgment is herewith made.

limb may wilt and become dry, but defoliation does not occur for a considerable period. Blighted twigs are characterized by the presence of elongate depressed lesions which girdle the branches below the dying parts.

On the trunk and larger branches, numerous cankers develop. The bark in cankered areas becomes dry and cracked, and sometimes the wood is exposed in the sharply demarcated cankers which result. Smaller cankers on the trunk are circular in outline, crateriform, and approximately one-quarter to one-half inch in diameter (FIG. 1); as many as a hundred may occur in a one foot-length of trunk. By the anastomosis of several smaller cankers, large ones several inches in length may arise. During the spring and early summer, an exudate attractive to ants accumulates at the surface of the lesions or flows from the cankered areas. The inner bark becomes reddish or purplish in color for a distance of several inches from the margin of the lesions, and short adventitious roots may form along the trunk.

One or more large limbs may die during a single growing season. Within another year or two, the cankers may become so abundant as to completely girdle the trunk, and within three or four years following the initial infection, the entire tree may be killed.

Examination of lesions on willow twigs with a hand lens will disclose the dark fungous stromata protruding from cracks in the outer bark. The peculiar white contents of fructifications characteristic of *B. Ribis* (see Shear, Stevens, and Wilcox (**6**), *pl. 9, fig. D*) may be seen by making a cut parallel to the surface and removing the bark, revealing the presence of from one to six locules within each stroma.

DESCRIPTION OF THE PATHOGEN

Only the pycnidial or *Dothiorella* stage of *B. Ribis* is present in lesions on willow twigs of the current season's growth. On older branches, perithecia may be found as well. Pycnidia and perithecia may be formed either in the same or in separate stromata. In the vicinity of Durham, N. C., both stages may be found at any time throughout the year. Fructifications of *B. Ribis,* fixed in formalin acetic alcohol, sectioned in paraffin to a thickness of 8 to 10 microns, and stained with Haidenhain's iron

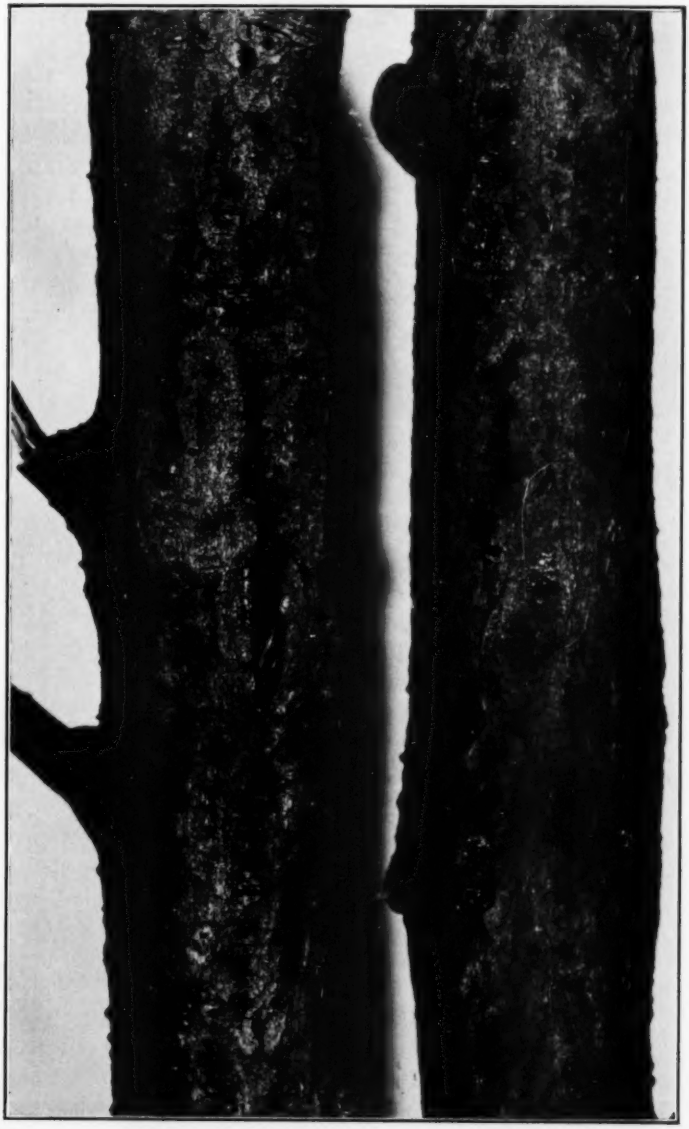

FIG. 1. Cankers caused by *B. Ribis* on willow branches. The large canker on the branch at the left is fissured at the margin and near the center. Small circular cankers have fused to make the large one.

alum hematoxylin, were used in making a morphological study of the pathogen.

PYCNIDIA.—On the inner wall of the pycnidium (FIG. 2) are thin-walled conidium-bearing cells, or conidiophores, each of which bears a sterigma-like process which projects inward from the pycnidial wall and also toward the ostiole. The conidia are abstricted singly from the apices of the conidiophores and are formed so profusely that, when the bark is wet, they may become aggregated at the opening of the ostiole to form white masses visible macroscopically. The conidia are unicellular, bluntly fusoid, and hyaline. They range in size from 18–30 × 4.5–8 μ, averaging 18–20 × 5–6 μ.

SPERMOGONIA.—Throughout the fall and winter, pycnidium-bearing stromata on willow may also contain locules in which are produced hyaline, oval to elongate microconidia, 2–3 × 1 μ. Similar structures were apparently observed on currant canes by Grossenbacher and Duggar (2), in "stromata bearing intact perithecia and often pycnidia." The uncertainty of Grossenbacher and Duggar concerning the nature of these structures may be seen from the following statement: "These *Cytospora*-like pycnidia seem to belong to a fungus that develops on the disintegrating stromata of the *Ribes* fungus; although that point remains uncertain and could not be tested culturally because the spores failed to germinate. Yet it is possible that this only represents a virescence of certain intact portions of old *Botryosphaeria* stromata."

Similar microconidial structures in *Botryosphaeria melanops* (Tul.) Wint. (*Dothidea melanops*) were illustrated many years ago by the Tulasnes (11). Since a considerable number of Ascomycetes are now known to possess spermogonia and spermatia of similar appearance, it seems reasonable to assume that the *Cytospora*-like structures observed by Grossenbacher and Duggar (2) on currant canes and which we have found on willow constitute the spermogonial stage of *B. Ribis*.

PERITHECIA.—Very little is known concerning the early stages in the development of the perithecia of *B. Ribis*. The locule is at first constituted of cells with dense protoplasmic contents. These cells later disintegrate, being utilized by the developing asci. Maturation of the asci, which are interspersed with numerous

paraphyses, proceeds centrifugally from the center of the perithecium, only a few of the asci reaching maturity at any given time (FIG. 3).

The thickened apical wall of the ascus is provided with a pore, which functions in the discharge of the ascospores. As the outer

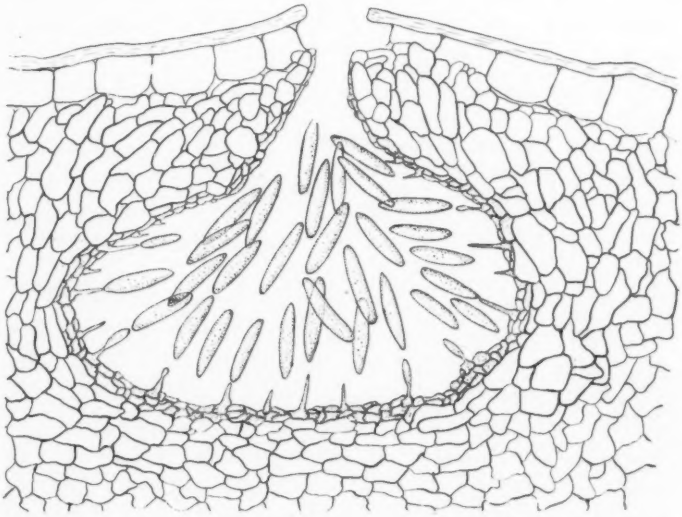

FIG. 2. Stroma in section showing pycnidium, conidiophoral cells and conidia.

ascus wall ruptures at the pore, the inner wall greatly elongates, and extends into the ostiole of the perithecium. Upon discharge of the ascospores, the wall of the ascus collapses, and the process of expulsion is repeated as succeeding groups of asci mature. The asci of *B. Ribis* on willow are broadly clavate to saccate, and measure 70–90 × 17–22 μ; the ascospores are hyaline, measuring 18–24 × 6–9 μ.

DEVELOPMENT OF CONIDIA AND ASCOSPORES

Although extended studies previously have been made concerning the host range and parasitism of *B. Ribis,* practically no attention has been devoted to the morphology and development of

the fungus since the pioneer work of Grossenbacher and Duggar (2). From specimens collected on willow, the formation of conidia and the development of the ascus and ascospores have been studied by means of paraffin sections of material fixed in formalin

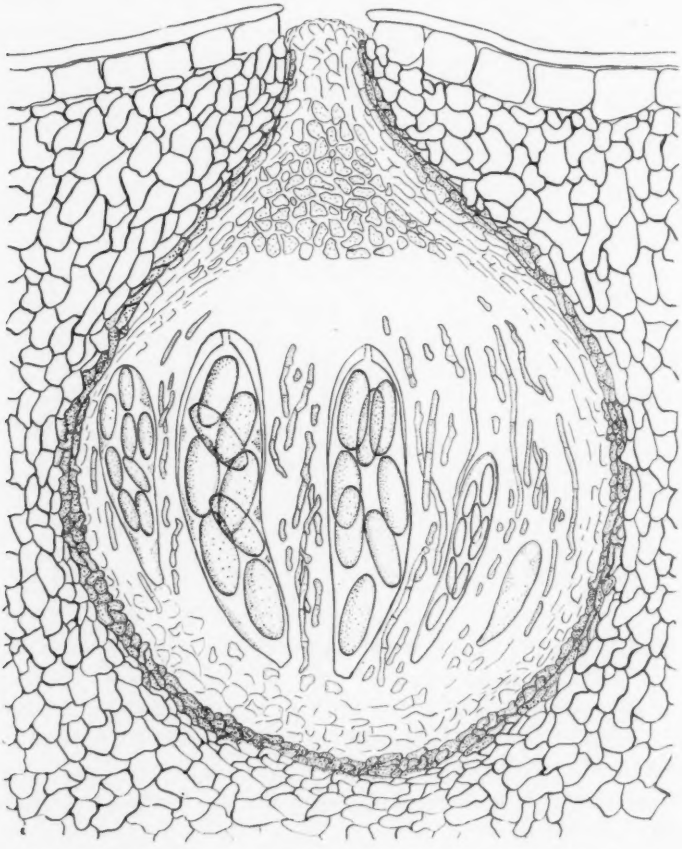

FIG. 3. Perithecial stroma in section with asci in different stages of maturity, and paraphyses.

acetic alcohol and stained with Haidenhain's iron alum hematoxylin.

CONIDIA.—The pycnidium is lined with a layer of thin-walled cells, some of which, herein called conidiophores, are to be con-

cerned with the formation of the conidia. Each conidiophore, which is at first uninucleate, develops an attenuate sterigma-like process (FIG. 15–16). Following a division of the conidiophore nucleus, one of the daughter nuclei remains in the basal portion of the conidiophore, the other migrating into the sterigma (FIG. 17). Then the distal end of the sterigma enlarges, further nuclear divisions occur within it (FIG. 18), and eventually a multinucleate conidium is abstricted (FIG. 19). The mature conidium contains on the average 4 to 6 nuclei. A single nucleus remains within the conidiophore, which may bear other conidia by a repetition of the process just described.

ASCUS AND ASCOSPORES.—Study of the development of the ascus and ascospores of *B. Ribis* was greatly facilitated by the fact that the asci do not mature simultaneously, a single perithecium containing, at a given time, asci of different ages. Very young asci 20–30 μ in length will contain a single large primary ascus nucleus (FIG. 4). Development proceeds as in other ascomycetous fungi, two, four, and finally eight free nuclei being formed (FIG. 5–7). When the ascus has attained about half of its final length, free cell formation occurs and eight uninucleate ascospores are delimited (FIG. 8, 9). Each ascospore nucleus then undergoes a series of divisions (FIG. 10–12) producing multinucleate ascospores. The mature ascospore while still within the ascus (FIG. 13, 14) may contain as many as 12 to 16 nuclei.

In order to ascertain the number of nuclei within each hyphal cell, conidia and ascospores were germinated on glass slides in a moist chamber, and the preparations of the germinating spores were stained *in toto* with hematoxylin. It was found by this means that practically the entire content of the spore becomes emptied into the germ tube, and that the germ tube attains a considerable length before cross walls are formed. The mycelium is therefore constituted of multinucleated cells for a period following germination and whatever the nuclear condition when stromata are being formed, the stromatic cells eventually are uninucleate.

CULTURAL CHARACTERS OF THE PATHOGEN

Monoconidial and monoascosporic isolations of *B. Ribis* from willow have been made. Monoconidial cultures were obtained by

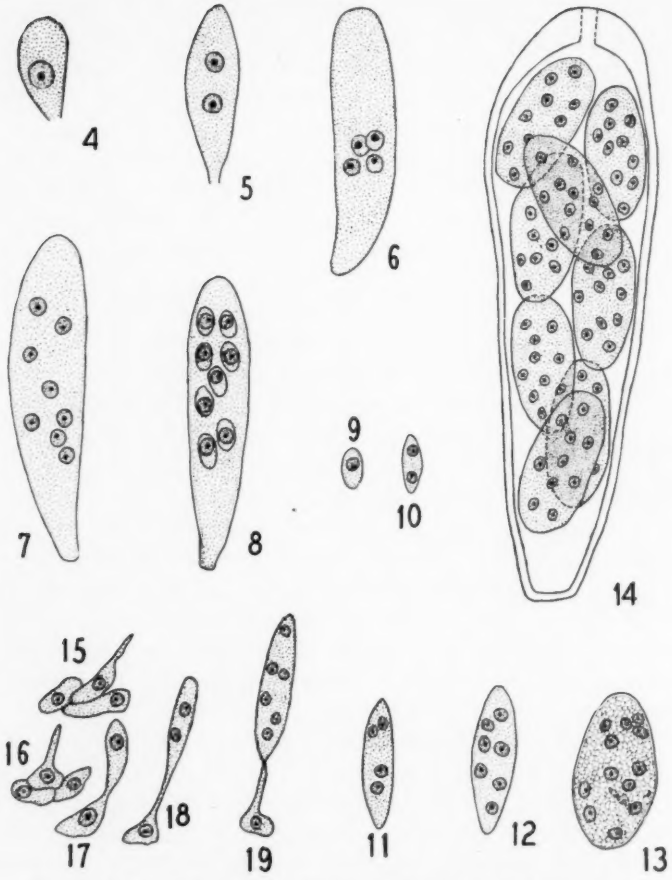

FIG. 4. Young ascus showing primary ascus nucleus; 5, binucleate ascus; 6, ascus in the 4-nucleate stage; 7, ascus containing 8 free nuclei; 8, uninucleate ascospores have been delimited; 9–13, stages in the transformation of uninucleate ascospores to multinucleate ascospores; 14, mature ascus, with pore in thickened apical portion; 15, 16, uninucleate conidiophoral cell of the *Dothiorella* stage and adjacent cells from the inner pycnidial wall; 17–19, stages in the formation and abstriction of conidia of the *Dothiorella* stage, and the formation of multinucleate conidia.

the dilution method, while cultures from single ascospores were initiated by inverting an agar plate over a lesion containing discharging asci. Both conidia and ascospores of *B. Ribis* germinate readily on malt agar or a medium composed of malt agar containing bits of sterilized willow twigs. Germ tubes several times the length of the spore are formed within 3 to 4 hours, and the entire surface of these media in Petri dish cultures is covered with a blackish-gray mycelium within 10 days.

Although Grossenbacher and Duggar (**2**) failed to induce sporulation in *B. Ribis,* from currant, in cultures upon a variety of media, the production of conidia from isolates from several hosts has been obtained by Stevens and Jenkins (**8**), Shear, Stevens, and Wilcox (**6**), and Smith (**7**). Pycnidial stromata of the *Dothiorella* type bearing an abundance of conidia indistinguishable from those on willow cankers were produced in our cultures derived from either ascospores or conidia.

Zonation occurred in cultures maintained under ordinary laboratory conditions of alternate light and darkness, but failed to occur in cultures grown in continuous total darkness. In addition to this zonation phenomenon, sectoring occurred in a few of the cultures, irrespective of the conditions of illumination. Occasionally, several sectorings occurred in a single culture, and sectors within sectors were produced in several instances. What relation sectoring may bear to the great variability of *B. Ribis* in nature, as shown by its wide host range, is as yet a matter of conjecture.

DISCUSSION

In nearly all species of Ascomycetes that have been investigated cytologically, the cells of the vegetative mycelium and of the reproductive structures, with the exception of the antheridia and ascogonia, are uninucleate. For this reason, the occurrence of a multinucleate condition in the mycelium, conidia, and ascospores of *B. Ribis* is of special mycological interest. In *Dipodascus albidus* Lagerh., *Monascus Barkeri* Dang., *Endomyces Magnusii* Ludw., and *Penicillium crustaceum* Link, the mycelium and conidia have been shown by Dangeard (**1**) to be multinucleate although the ascospores are uninucleate. In *Aspergillus flavus* (L.) Link (*Eurotium herbariorum* Wigg.), the mycelial cells, phialides, conidia, and ascospores are all coenocytic (**1**).

Although the conidia and ascospores of *B. Ribis* are multinucleate, all of the nuclei of a single conidium or ascospore have the same origin and hence are homocaryotic or genetically similar. In the case of certain other fungi, as *Botrytis cinerea* Pers., it has been demonstrated (Hansen & Smith, **3**) that the multinucleate spores are heterocaryotic, this condition having arisen from hyphal anastomoses and nuclear migrations. Hansen and Smith suggest that the variability of *Botrytis cinerea* and perhaps of other fungi may be due to nuclear heterogeneity. Whether any significance can be attached to the multinucleate condition in *Botryosphaeria Ribis* in the light of its adaptability to a wide host range in nature and its great capacity for variation in culture is entirely conjectural.

SUMMARY

A disease of willow caused by *Botryosphaeria Ribis* is characterized by the presence of cankers on the twigs, larger branches, and trunk. Successive blighting of the branches eventually results in the death of the tree.

A single stroma of this organism on willow may simultaneously bear conidia, microconidia and ascospores. The microconidia are probably spermatia. Cultures derived from conidia are similar to those originating from ascospores, and from both, the *Dothiorella* stage may be produced.

The development of the conidia, asci and ascospores of *B. Ribis* has been studied cytologically. The conidia are multinucleate but are borne by uninucleate conidiophoral cells. The development of the nuclei within the ascus is like that of other Ascomycetes. The ascospores are uninucleate when delimited but become multinucleate as they mature. For a period following germination the hyphal cells also are multinucleate.

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10. **Stevens, N. E.** Occurrence of the currant cane blight fungus on numerous hosts in the southern states. Mycologia **18**: 278–282. 1926.
11. **Tulasne, L. R. & Tulasne, C.** Selecta fungorum carpologia. II. 392 pp. 1863 (see pp. 68–70).

A NOTE ON THE OCCURRENCE OF MARASMIUS PYRINUS

VERA K. CHARLES

(WITH 1 FIGURE)

A fungus of rare occurrence or at least one rarely collected was recently submitted to the Division of Mycology and Disease Survey of the Bureau of Plant Industry for determination. The fungus occurred on the fruit of apple, and was so small, only about 1 mm. in diameter, that it suggested a species of *Stilbum*. However with the aid of a hand lens, it was discovered to be an agaric. The collection was received from A. B. Groves, Winchester, Va., with the information that he had observed it several times before, and that it always seemed to follow arsenical spray injury. In the present case, the fungus developed on a definite spot which had resulted from the use of an arsenical spray.

A study of the material showed the fungus to be a species of *Marasmius, Marasmius pyrinus* described by Ellis.[1] The type material occurred on leaves of *Pyrus communis*, but the species has been reported on several different hosts. The Mycological Collections contain the following material:

A specimen of Ellis, N. Am. Fungi *401* evidently part of the type dated June 1880.

A second specimen of Ellis on old *Lactuca*, bearing the date 1899. This latter specimen was evidently first determined as *Marasmius* sp., and the specific name was later added to the label. This seems to be the case as the two words, the generic and specific names, are separated, and the ink is different. The specimen on *Lactuca* was given to the Mycological Collections by Dr. C. L. Shear, who had purchased certain remainders of the Ellis Herbarium. The Mycological Collections also contain specimens determined as *Marasmius pyrinus* on cankered apple bark; the cankers were probably due to bacterial blight. This material was

[1] Ellis, J. B. New species of North American fungi. Bull. Torrey Club **8**: 64. 1881.

FIG. 1. *Marasmius pyrinus* on the fruit of apple, × 6.

collected at Rockville, Md., in 1920 by L. M. Hutchins and referred to the Office of Mycology for determination. It is typical except in the size of the pileus which exceeds the measurements given by Ellis for this species, being 2–2.5 mm. in diameter in-

stead of 1–1.5 as given in the original description of this species by Ellis.

Another specimen contained in the Mycological Collections was collected and determined by G. W. Carver, of the Tuskegee Institute, Aug. 23, 1930. This material is on *Ligustrum* sp., and is more typical in size, but lighter in color than the collections on apple.

Pennington [2] in his treatment of *Marasmius* suggests that *M. pyrinus* Ellis may be synonymous with *M. capillipes* described by Saccardo [3] in 1876 on *Pyrus communis*. Probably Pennington did not have the type or adequate material for examination and comparison necessary to make this identity certain. The absence of good material for comparison is also the difficulty in the present study. The Mycological Collections do not contain a type specimen of *Marasmius capillipes*. Furthermore, a Saccardo specimen of this species on leaves of *Fraxinus ornus* Sacc., Mycotheca italica No. 2, published in 1897, unfortunately shows no trace of the fungus. In view of this situation it would seem best to retain Ellis' name for the fungus as it occurs in the United States.

Although this species is extremely minute, the characters noted in Ellis' description particularly the rugoso-sulcate pileus, due to the spiny cells and visible by the aid of a hand lens, renders the species fairly easy of recognition. The accompanying illustration of the fungus on the apple near the stem shows this condition.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

[2] Pennington, L. H. N. Am. Flora **9**: 281. 1915.

[3] Saccardo, P. A. Fungi veneti novi vel critici Ser. V. Nuovo Giorn. Bot. Ital. **8**: 162. 1876.

NOTES AND BRIEF ARTICLES

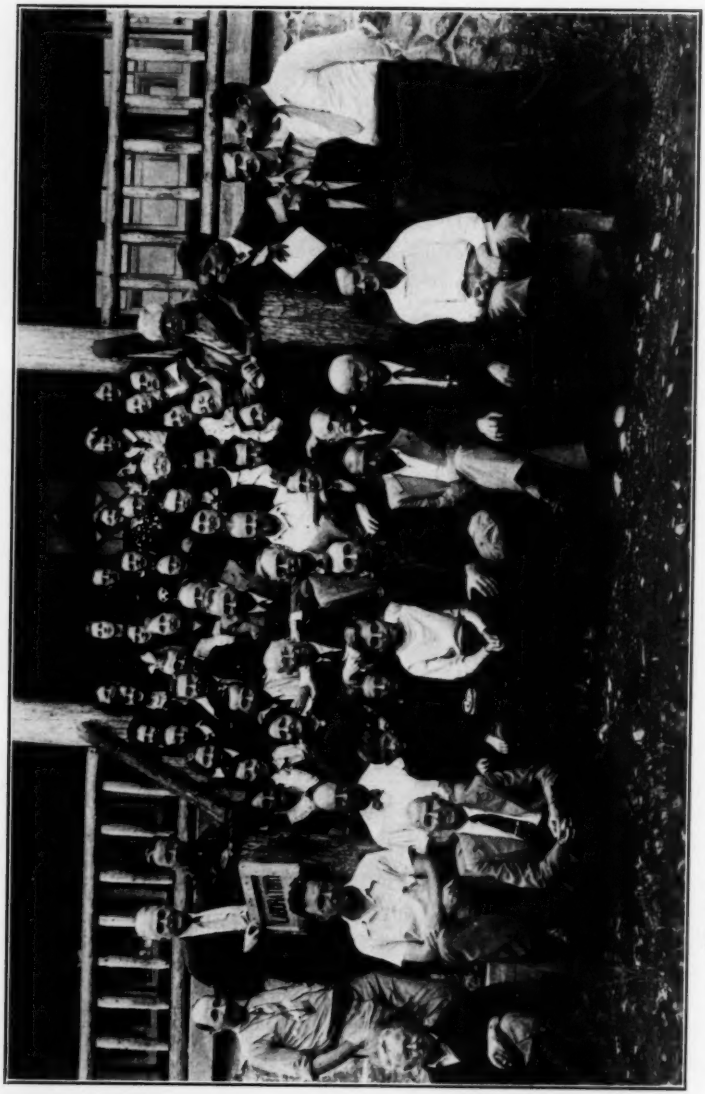
List VI of "Fungus Specimens Available for Exchange" has been issued under date of August 1, 1938, by the Division of Mycology and Disease Survey, of the Bureau of Plant Industry, Washington, D. C. This list is a revision of and supersedes previous lists. A copy will be sent to any mycologist or mycological institution interested in the exchange of fungus material.—JOHN A. STEVENSON.

Motions dealing with Nomenclature for consideration by the VIIth International Botanical Congress, Stockholm 1940, should be sent before July 1, 1939, to the Rapporteur general, Dr. T. A. Sprague, The Herbarium, Royal Botanic Gardens, Kew, Surrey, England.

Motions must be presented in the form of additional articles, or amendments, to the International Rules. They should be drafted as briefly as possible. At least 100 printed copies must be presented.—JOHN A. NANNFELDT.

CANTHARELLUS MULTIPLEX AGAIN

In the May–June number of MYCOLOGIA for 1937 and again in the July–August number for 1938 (pp. 286 and 372, respectively) there appeared short articles on the finding of *Cantharellus multiplex* Underwood. The first of these articles detailed its collection in two localities in Quebec; the second relates to its discovery in Middle Boulder Canyon, Colorado. The authors of both of these articles have overlooked Kauffman's published notes and photo of this species (Papers Mich. Acad. Sci., Arts, Let. **5**: 124. pl. V. 1925). Kauffman infers that he at that time had already collected it in the Rocky Mountains of Wyoming and Colorado, and in the Cascade Mountains of Washington and Oregon. He remarks on its similarity to *Cantharellus clavatus* and concludes that it is only "a very extreme growth condition" of that species. There would seem to be no warrant for transferring the species



MYCOLOGICAL SOCIETY OF AMERICA, SUMMER FORAY, AUGUST 24–27, DUCHESNAY, QUEBEC.

to the genus *Craterellus* as was done by the author of one of the above-cited papers.—L. O. OVERHOLTS.

MYCOLOGICAL SOCIETY OF AMERICA

REPORT ON THE 1938 FORAY

(WITH 1 FIGURE)

The 1938 Summer Foray of the Mycological Society of America was held at Duschesnay, Quebec, August 23–27, at the Forest Rangers School. Over fifty persons attended. Adequate laboratory facilities were supplied for studying and drying specimens and a new dormitory was rushed to completion affording comfortable quarters. The plans of the foray were well arranged by Dr. Rene Pomerleau. Trips to points of mycological interest were arranged for each morning. The afternoons were utilized for studying and discussing specimens or individual collecting. Excellent collecting occurred in the immediate vicinity.

On Tuesday evening the Honorable J. Bourque, Minister of Lands and Forests welcomed the Society. On Wednesday evening colored slides of fungi were shown by Robert Hagelstein and E. B. Mains, and Henry A. C. Jackson exhibited his excellent paintings of agarics. On Friday evening films illustrating the wild life and picturesque localities of Quebec were shown.

Due to frequent rains throughout August fungi were abundant. Arrivals on Tuesday drove through a steady downpour. On Wednesday the weather cleared and ideal mycological conditions prevailed. This together with easy accessibility and variety of habitats resulted in an accumulation of specimens that taxed all the drying facilities. A number of rare and interesting species were found.

A business meeting was held on Thursday evening at which President L. O. Overholts presided. Invitations were received to hold the 1939 foray in Florida and in Louisiana. Other possibilities were discussed with the White Mountains and Rhode Island being favored. Due to the small amount of published information in regard to the mycological flora of Quebec, it was voted to

publish a complete list of the fungi collected during the foray. A committee consisting of L. O. Overholts, Rene Pomerleau and E. B. Mains was appointed to supervise the preparation of the list. After voting that the thanks of the Society be extended to the Honorable J. Bourque, Director Henri Roy, Dr. Rene Pomerleau and the local committee for the excellent facilities, the meeting adjourned.

The foray ended on Saturday with a trip to the Laurentian National Park. At Camp Mercier, the group was served a delicious lunch with brook trout direct from the lake.—E. B. MAINS.

Members who have not yet paid their dues are requested to send them to Dr. J. N. Couch, Department of Botany, University of North Carolina, Chapel Hill, North Carolina. By cooperating in this manner they will greatly assist the Secretary-Treasurer and will avoid undue delay in receiving MYCOLOGIA.—D. H. LINDER.

The Summer Foray of the Mycological Society of America will be held in the Great Smoky Mountains during the latter part of August. The exact time and the location of headquarters will be announced at a later date. However, it would greatly facilitate arrangements if all those who think they may attend would be so kind as to notify David H. Linder, The Farlow Herbarium, 20 Divinity Ave., Cambridge, Mass.—D. H. LINDER.

LATIN DIAGNOSES

Since the adoption, by the International Commission on Nomenclature, of the rule requiring that the descriptions of all new species and genera be accompanied by Latin diagnoses, most journals, including MYCOLOGIA, have required their contributors to comply with this regulation. If this is not done, such species and genera are not legally published, and if later translated into Latin, the date and place of publication will be to the work containing such Latin diagnoses. Without attempting to discuss the merits of

this requirement, failure to comply would at least result in much confusion.

This requirement, on the part of the Mycological Society, as a prerequisite for publication in MYCOLOGIA, has aroused such a storm of protest from some members of the Society that the matter has been reconsidered, the Society voting to leave the question in the hands of the Editorial Board. After careful consideration the Board has decided to make this a recommendation rather than a requirement. This does not mean that the Board shall not still continue to request such diagnoses. In fact, if they are not furnished by the author, it will be necessary for the Editor to solicit these in each individual case, which will involve not only extra work but delay in the publication of papers. If you wish to avoid such delay, send *brief* Latin diagnoses with all descriptions of new species or genera when manuscript is submitted for publication.—FRED J. SEAVER, EDITOR.

MYCOLOGIA ENDOWMENT FUND

During the year 1938, the restricted Mycologia Endowment Fund has been increased by the addition of one thousand dollars ($1000), bringing the total to six thousand dollars ($6000). All of this has been built up either through private donation for that purpose, or through money secured from the sale of the early volumes (1–24). It has been the purpose of the Managing Editor to pay all the current expenses of MYCOLOGIA out of the current receipts, leaving the money from the sale of the early volumes to be added to this fund. Few mycologists are able to assist directly in the building up of such an endowment. However, any member of the Society may use his influence to place the early volumes of MYCOLOGIA where they are needed, and in this way indirectly contribute to the building up of this fund. It is intended that the interest on this endowment will eventually be used to promote special features in the journal which cannot be taken care of out of the current income. To this end your cooperation is solicited.—FRED J. SEAVER, MANAGING EDITOR.

SAWADA'S DISCOVERY OF ACHLYA FLAGELLATA AS A PARASITE OF FISH

In a previous paper (Tiffney and Wolf, **1937**), it was reported that the water mold *Achlya flagellata* Coker is capable of infecting the newt, *Triturus viridescens,* as well as the fishes *Lebistes reticulatus, Fundulus heteroclitus,* and *Salvelinus fontinalis.* The author wishes to correct a most unfortunate error in this paper; namely, the statement that *A. flagellata* had never previously been shown to parasitize fishes.

Sawada (**1912**) published entirely in Japanese the results of a study of a disease of paddy rice seedlings in Formosa, caused by a fungus which he called *Achlya prolifera* (Nees) de Bary. Incidental to the phytopathological portion of this investigation, Sawada (**1912, 1919**) reported that this fungus was found to occur under natural conditions on living goldfish (*Carassius aurantus* var.) and the Japanese fighting fish. Healthy goldfish and fighting fish, as well as Medaka (*Oryzias latipes*) and Gusai-chi, when placed in water containing cultures of the fungus, became covered with the cottony mycelium, and were killed within a week. Sawada (**1912**) published an excellent photograph of a fighting fish killed by the fungus (*Pl. IV, fig. 1*) showing tufts of mycelium on the head, gills, fins, and tail. A complete diagnosis and several carefully drawn plates of the fungus are also included in Sawada's paper.

According to Nagai (**1931**), Cejp (**1934**), and Coker and Matthews (**1937**), the fungus which Sawada called *A. prolifera* (Nees) de Bary is not that species, but is probably *A. flagellata* Coker. The writer has compared Sawada's description and plates with those of *A. flagellata,* and is also of the opinion that the two fungi are identical. It would appear, therefore, that Sawada must be credited with the discovery of the fact that *A. flagellata* is capable of parasitizing fishes, even though this species was not described for more than a decade after the publication of his observations.

This brief note was written during the tenure of a National Research Fellowship under the direction of Prof. Wm. H. Weston, Jr., to whom the author is indebted for the opportunity of studying an authorized abridged translation of Sawada's paper.

FRED T. WOLF.

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Sawada, K. 1912. Investigations of the Paddy Seedling Decay in Formosa. Special Bull. *3*, Agr. Expt. Sta., Govt. of Formosa, 84 pp., 10 pl. (Japanese).

——. 1919. Descriptive catalogue of Formosan fungi. Special Bull. *19*, Agr. Expt. Sta., Govt. of Formosa, pp. 44–55 (Japanese).

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